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CONTENTS—PART 3

	Page
Program Committee	iv
Chronological List of Sessions	v
Thematic List of Sessions	xi
Abstracts in Session Order*	
Friday, November 20-Saturday, November 21	1493-1724
Key Word Index	1725
Author Index	1933

*6,679 volunteer abstracts, 19 symposium and workshop abstracts, 2 special lecture abstracts.

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CHRONOLOGICAL LIST OF SESSIONS

(see page xi for Thematic List of Sessions)

Session Number and Title	Page
MONDAY	
Public Lecture—8:00 p.m.	
1. Aging and Alzheimer's Disease: New Light on Old Problems. P. Davies	No abstract
TUESDAY	
Workshop—8:30 a.m.	
2. Chemical Architecture of the Cerebellar Cortex: Structure and Function. *Chaired by:* J.S. King	1
Symposium—8:30 a.m.	
3. A Neural Systems Approach to the Analysis of Fear and Anxiety. *Chaired by:* M. Davis	1
Workshop—8:30 a.m.	
4. Visceral Afferents: Signaling and Integration. *Chaired by:* M. Kalia and L. Kruger	1
Slide Sessions—8:30 a.m.	
5. Visual cortex I	2
6. Process outgrowth I	5
7. Neuronal death I	8
8. Control of posture and movement I	11
9. Feeding and drinking I	14
10. Neuroendocrine controls: pituitary I	17
11. Peptides: biosynthesis, metabolism and biochemical characterization I	20
12. Retina I	23
13. Basal ganglia I	27
14. Monoamines and behavior I	30
15. Gene structure and function I	33
16. Transmitters in invertebrates I	37
17. Auditory system I	40
Poster Sessions—8:30 a.m.	
18. Interhemispheric relations	43
19. Biological rhythms I	48
20. Hormonal control of behavior I	53
21. Spinal cord and brainstem I	59
22. Pharmacology of synaptic transmission I	63
23. Pharmacology of synaptic transmission II	67
24. Neurotoxicity: MPTP and ethanol	71
25. Sensory systems: somatosensory	75
26. Sensory systems: auditory, olfactory, gustatory	79
27. Chronic drugs and neurotoxicity I	83
28. Chronic drugs and neurotoxicity II	88
29. Action potentials and ion channels I	91
30. Action potentials and ion channels II	95
31. Action potentials and ion channels III	99
32. Action potentials and ion channels IV	104
33. Pain modulation I	108
34. Pain: central pathways I	113
35. Glia I	117
36. Axonal and intracellular transport	121
37. Brain metabolism I	125
38. Comparative neuroanatomy: fish and below	130
39. Peptides: opiate receptors	134
40. Invertebrate sensory processing I	137
41. Invertebrate sensory processing II	140
42. Drug effects on receptors I	143
43. Drug effects on receptors II	146
44. Postsynaptic mechanisms I	150
45. Postsynaptic mechanisms II	154
46. Transplantation I	159
47. Neural plasticity in adult animals: spinal cord	164
48. Sprouting and sprouting mechanisms I	167
49. Motor systems and sensorimotor integration: oculomotor system I	170
Presidential Special Lecture—11:30 a.m.	
50. The Neurology of Memory. L.R. Squire	No abstract
Workshop—1:00 p.m.	
51. Cocaine: Modulation of Monoamine Function. *Chaired by:* J.M. Lakoski	175
Symposium—1:00 p.m.	
52. The Basics of Molecular Biology. *Chaired by:* J.L. Roberts	175
Slide Sessions—1:00 p.m.	
53. Action potentials and ion channels V	175
54. Excitatory amino acids: pharmacology I	178
55. Cell lineage and differentiation I	181
56. Trophic agents: nerve growth factor I	184
57. Somatic afferents I	187
58. Dopamine receptors	190

Session Number and Title	Page
59. Glia II	193
60. Neuroendocrine controls: pituitary II	196
61. Receptor regulation I	199
62. Subcortical visual pathways I	202
63. Learning and memory: anatomy I	205
64. Opiates, endorphins and enkephalins: physiological effects I	208
65. Biological rhythms II	211
66. Transmitters and receptors in disease I	214
Poster Sessions—1:00 p.m.	
67. Monoamines and behavior II	217
68. Hormonal control of behavior II	222
69. Motor systems and sensorimotor integration: cerebellum I	228
70. Transmitters in invertebrates II	233
71. Visual system: development and plasticity I	237
72. Cortex: motor cortex I	241
73. Somatosensory cortex and thalamocortical relationships I	247
74. Morphogenesis and pattern formation I	251
75. Process outgrowth II	255
76. Sleep	260
77. Acetylcholine: regulation	266
78. Regulation of autonomic function I	269
79. Cardiovascular regulation: CNS pathways I	275
80. Cardiovascular regulation: CNS pathways II	280
81. Transplantation II	285
82. Regeneration I	290
83. Peptides: anatomical localization I	295
84. Pain modulation II	299
85. Comparative neuroanatomy: amphibians, reptiles and birds	304
86. Presynaptic mechanisms I	308
87. Synaptic structure and function I	313
88. Synaptic structure and function II	316
89. Auditory system II	320
90. Auditory system III	325
91. Auditory system IV	328
92. Feeding and drinking II	332
93. Alcohol and barbiturates I	337
94. Serotonin: functional studies I	342
95. Control of posture and movement II	346
96. Control of posture and movement III	352
Special Lecture—4:00 p.m.	
97. Neural and Peripheral Factors Regulating Function and Growth of the Adenohypophysis. W.W. Vale	No abstract
Presidential Symposium—8:00 p.m.	
98. Selective Attention and Its Neural Basis. *Chaired by:* M. Mishkin	No abstract

WEDNESDAY

Session Number and Title	Page
Symposia—8:30 a.m.	
99. Proto-Oncogenes in the Nervous System. *Chaired by:* M.R. Hanley	357
100. Peptide-Monoamine Interactions: From Molecular Mechanisms to Behavior. *Chaired by:* T.D. Reisine	357
Slide Sessions—8:30 a.m.	
101. Visual cortex II	357
102. Chemical sensory systems I	360
103. Epilepsy I	363
104. Process outgrowth: growth cone behavior	366
105. Control of posture and movement IV	370
106. Synaptogenesis I	373
107. Messenger RNA regulation I	376
108. Retina II	379
109. Excitatory amino acids: receptors I	382
110. Regulation of autonomic function II	385
111. Invertebrate learning and behavior I	388
112. Motor systems and sensorimotor integration: oculomotor system II	391
113. Regeneration: spinal cord I	394
114. Neuroethology I	397
Poster Sessions—8:30 a.m.	
115. Neuroendocrine controls: other I	401
116. Monoamines and behavior III	405
117. Disorders of motor systems and neural prostheses I	410
118. Peptides: substance P receptors	412
119. Neuroendocrine controls: pituitary III	414
120. Biological rhythms III	419
121. Neuropeptides and behavior I	424
122. Subcortical visual pathways II	428
123. Aging and dementia: transmitters	433
124. Aging and dementia: function I	439
125. Basal forebrain and cholinergic pathways	443
126. Behavioral pharmacology: dopamine	447
127. Psychotherapeutic drugs: anxiolytics	452
128. Psychotherapeutic drugs: antipsychotics	457
129. Feeding and drinking III	462
130. Process outgrowth III	467
131. Somatosensory cortex and thalamocortical relationships II	470
132. Somatosensory cortex and thalamocortical relationships III	472
133. Catecholamines I	475
134. Catecholamines II	479
135. Acetylcholine: muscarinic receptors	484
136. Dopamine receptor functions	488
137. Diseases of the nervous system I	492
138. Diseases of the nervous system II	497
139. Alcohol and barbiturates II	501

Session Number and Title	Page
140. Alcohol and barbiturates III	506
141. Transplantation III	511
142. Trophic agents: nerve growth factor II	516
143. Trophic agents: nerve growth factor III	518
144. Spinal cord I	521
145. Subcortical somatosensory pathways: trigeminal	523
146. Comparative neuroanatomy: mammals, etc.	525
147. Action potentials and ion channels VI	530
148. Action potentials and ion channels VII	535
149. Auditory system V	538
150. Auditory system VI	543
151. Auditory system VII	547
152. Trophic agents: nerve growth factor IV	550
153. Feeding and drinking IV	553
154. Gene structure and function II	557
155. Peptides: receptors	561
156. Transmitters and receptors in disease II	564
Presidential Special Lecture—11:30 a.m.	
157. Cortical Computational Maps for Auditory Imaging. N. Suga	No abstract
Symposia—1:00 p.m.	
158. The Role of Nectins (Cell Binding Molecules) in Neural Development. *Chaired by:* L. Glaser	567
159. Structural Computer Simulations in Developmental and Systems Neurobiology. *Chaired by:* J.M. Bower	567
Slide Sessions—1:00 p.m.	
160. Transplantation IV	568
161. GABA and benzodiazepine: pharmacology I	570
162. Trophic interactions I	573
163. Action potentials and ion channels VIII	576
164. Pain: central pathways II	580
165. Messenger RNA regulation II	583
166. Feeding and drinking V	586
167. Visual system: development and plasticity II	589
168. Development of invertebrates I	592
169. Presynaptic mechanisms II	595
170. Behavioral pharmacology: dopamine and neuroleptics	598
171. Motor systems and sensorimotor integration: cerebellum II	601
172. Biological rhythms IV	604
173. Learning and memory: physiology I	607
Poster Sessions—1:00 p.m.	
174. Neuropeptides and behavior II	611
175. Invertebrate learning and behavior II	615
176. Neuroethology II	620
177. Visual cortex III	624
178. Visual cortex IV	629
179. Motor systems and sensorimotor integration: vestibular system I	634
180. Opiates, endorphins and enkephalins: anatomy and chemistry I	637
181. Learning and memory: anatomy II	642
182. Metabolic studies	647
183. Human behavioral neurobiology I	650
184. Learning and memory: pharmacology I	654
185. Monoamines and behavior IV	659
186. Neuroendocrine controls: pituitary IV	664
187. Neurotransmitters: uptake, storage and secretion I	668
188. Cortex: motor cortex II	672
189. Staining and tracing techniques I	676
190. Staining and tracing techniques II	679
191. Autonomic nervous system	682
192. Endocrine control of development I	687
193. Nutritional and prenatal factors I	691
194. Neurotoxicity: metals	695
195. Cell lineage and differentiation II	699
196. Interactions between neurotransmitters I	703
197. Characterization of cholinergic receptors I	706
198. Regional localization of receptors and transmitters I	710
199. Control of posture and movement V	713
200. Development and plasticity: aging	718
201. Receptor regulation II	723
202. Receptor regulation: cholinergic	726
203. Regulation of autonomic function III	728
204. Regulation of autonomic function IV	733
205. Cardiovascular regulation: heart, blood flow, nerves	739
206. Cardiovascular regulation: hypertension	744
207. Regeneration: spinal cord II	748
208. Excitatory amino acids: mechanisms	751
209. Excitatory amino acids: receptors II	756
210. Excitatory amino acids: pharmacology II	761
211. Opiates, endorphins and enkephalins: physiological effects II	766
212. Blood-brain barrier I	771
213. Histochemical methods	776
214. Somatic afferents II	777
Special Lecture—4:00 p.m.	
215. Excitatory Synaptic Transmission in the Hippocampus. C.F. Stevens	782
Grass Foundation Lecture—8:00 p.m.	
216. Molecular Basis of Axonal Transport—Kinesin and Other Transport Proteins. T.S. Reese	No abstract

THURSDAY

Symposium—8:30 a.m.	
217. Molecular Genetic Analysis of Neuronal Development. *Chaired by:* C.S. Goodman	783
Workshop—8:30 a.m.	
218. Information Processing in the Macaque Retina. *Chaired by:* R.M. Shapley	783

Session Number and Title	Page
Slide Sessions—8:30 a.m.	
219. Transplantation for movement disorders	783
220. Neurotoxicity I	786
221. Modulators I	789
222. Action potentials and ion channels IX	792
223. Characterization of cholinergic receptors II	795
224. Serotonin: functional studies II	799
225. Learning and memory: anatomy III	802
226. Catecholamines III	805
227. Cardiovascular regulation: CNS pathways III	808
228. Brain metabolism II	811
229. Invertebrate learning and behavior III	814
230. Aging and dementia: molecular biology I	818
231. Circuitry and pattern generation I	821
232. Spinal cord and brainstem II	824
Poster Sessions—8:30 a.m.	
233. Neuroendocrine controls: pituitary V	827
234. Monoamines and behavior V	830
235. Learning and memory: pharmacology II	834
236. Learning and memory: physiology II	839
237. Learning and memory: pharmacology III	843
238. Human behavioral neurobiology II	848
239. Spinal cord and brainstem III	852
240. Gene structure and function III	856
241. Subcortical visual pathways III	860
242. Biological rhythms V	864
243. Neuroethology III	868
244. Control of posture and movement VI	872
245. Feeding and drinking VI	877
246. Psychotherapeutic drugs: antidepressants	882
247. Amygdala and limbic cortex	885
248. Cell lineage and differentiation III	888
249. Adrenergic receptors	893
250. Cyclic nucleotides I	898
251. Biogenic amines: toxins	903
252. Catecholamines: electrophysiology	908
253. Catecholamines IV	913
254. Opiates, endorphins and enkephalins: physiological effects III	915
255. Neuronal death II	920
256. Transmitter phenotypic plasticity	924
257. Biochemical and pharmacological correlates of development I	926
258. Neurotoxicity II	930
259. Interactions between neurotransmitters II	934
260. Characterization of neuronal nicotinic cholinergic receptors	937
261. Epilepsy: human and genetic models	941
262. Epilepsy: kindling	946
263. GABA and benzodiazepine: anatomy and cytochemistry	951
264. GABA and benzodiazepine: pharmacology II	955
265. GABA and benzodiazepine: receptor binding	960
266. GABA and benzodiazepine receptors: molecular characterization	964
267. GABA and benzodiazepine receptors: behavioral studies	966
268. Regeneration: lower forms	971
269. Neural plasticity in adult animals: hippocampus	974
270. Basal ganglia: electrophysiology and behavior	978
271. Subcortical somatosensory pathways	983
272. Pain modulation III	988
273. Peptides: anatomical localization II	992
274. Behavioral pharmacology I	997
History of Neuroscience Lecture—11:30 a.m.	
275. The Ontogeny of Experimental Neuroembryology. V. Hamburger	No abstract
Symposia—1:00 p.m.	
276. Neuropeptides/Neural Systems in Fever, Inflammation and Immune Responses. *Chaired by:* J.M. Lipton	1001
277. Dynamic Changes of Synaptic Structure under Normal and Experimental Conditions. *Chaired by:* D. Purves	1001
Slide Sessions—1:00 p.m.	
278. Opiates, endorphins and enkephalins: physiological effects IV	1001
279. Postsynaptic mechanisms III	1004
280. Neural plasticity in adult animals I	1007
281. Action potentials and ion channels X	1010
282. Trophic agents: nerve growth factor and others	1013
283. Pain modulation IV	1016
284. Peptide: receptors I	1019
285. Visual system: development and plasticity III	1023
286. Acetylcholine: metabolism I	1026
287. Excitotoxins I	1029
288. Cardiovascular regulation: CNS pathways IV	1032
289. Behavioral pharmacology II	1035
290. Biological rhythms VI	1038
291. Regeneration II	1040
Poster Sessions—1:00 p.m.	
292. Visual cortex V	1043
293. Retina III	1049
294. Retina IV	1054
295. Invertebrate motor function	1059
296. Reflex function I	1062
297. Learning and memory: anatomy IV	1065
298. Transmitters in invertebrates III	1070
299. Peptides: biosynthesis, metabolism and biochemical characterization II	1073
300. Transmitters and receptors in disease III	1077
301. Neuroendocrine controls: pituitary VI	1081
302. Messenger RNA regulation III	1085
303. Motor systems and sensorimotor integration: oculomotor system III	1090
304. Cortex: prefrontal and premotor areas	1095
305. Learning and memory: physiology III	1100
306. Neuroendocrine controls: other II	1104
307. Neurotransmitters: uptake, storage and secretion II	1109
308. Morphogenesis and pattern formation II	1114
309. Cell lineage and differentiation IV	1119
310. Learning and memory: anatomy V	1123

Session Number and Title	Page
311. Regional localization of receptors and transmitters II	1128
312. Modulators II	1132
313. Biochemical and pharmacological correlates of development II	1136
314. Development of invertebrates II	1139
315. Limbic system	1145
316. Aging and dementia: plaques, tangles, amyloid	1149
317. Epilepsy: brain slices	1154
318. Epilepsy II	1159
319. Hypothalamus	1163
320. Feeding and drinking VII	1168
321. Control of posture and movement VII	1173
322. Catecholamines: cell culture	1178
323. Serotonin receptors: radioligand binding studies	1181
324. Receptor regulation: neuropeptides	1185
325. Acetylcholine: localization	1188
326. Acetylcholine: metabolism II	1191
327. Acetylcholine: choline uptake	1195
328. Dopamine receptors: ligand binding and purification	1197
329. Pain: central pathways IV	1199
330. Glia III	1202
331. Regeneration: PNS	1206
332. Muscle I	1212
333. Muscle II	1216
Special Lecture—4:00 p.m.	
334. Neuroethology of Electric Fish: The Role of Analogies versus Models in Comprehending Neural Systems. T.H. Bullock	1219

FRIDAY

Symposia—8:30 a.m.	
335. Stimulant-Induced Sensitization—Behavior and Neuropharmacology. *Chaired by:* W.H. Riffee	1220
336. A Heart-Brain Peptide: The Atrial Natriuretic Factor. *Chaired by:* J.M. Saavedra	1220
Slide Sessions—8:30 a.m.	
337. Process outgrowth IV	1220
338. Motor systems and sensorimotor integration: vestibular system II	1223
339. Biochemical and pharmacological correlates of development III	1226
340. GABA and benzodiazepine: receptors	1229
341. Neural plasticity in adult animals II	1232
342. Serotonin	1235
343. Presynaptic mechanisms III	1238
344. Visual system: development and plasticity IV	1241
345. Cardiovascular regulation: CNS pathways V	1244
346. Opiates, endorphins and enkephalins: anatomy and chemistry II	1247
347. Blood-brain barrier II	1249
348. Diseases of the nervous system: viral and traumatic injury to CNS	1252
349. Transmitters in invertebrates IV	1255
350. Auditory system VIII	1258
Poster Sessions—8:30 a.m.	
351. Motor systems and sensorimotor integration: cerebellum III	1261
352. Clinical CNS neurophysiology I	1264
353. Clinical CNS neurophysiology II	1268
354. Interactions between neurotransmitters III	1271
355. Peptides: biosynthesis, metabolism and biochemical characterization III	1275
356. Peptides: physiological effects I	1278
357. Messenger RNA regulation IV	1283
358. Regional localization of receptors and transmitters III	1288
359. Stress, hormones and the autonomic nervous system I	1292
360. Retina V	1297
361. Opiates, endorphins and enkephalins: physiological effects V	1302
362. Peptides: physiological effects II	1307
363. Motor systems and sensorimotor integration: vestibular system III	1312
364. Learning and memory: physiology IV	1315
365. Motivation and emotion I	1320
366. Aging and dementia: molecular biology II	1324
367. Hippocampus	1329
368. Catecholamines: MPTP	1334
369. Catecholamines: anatomical studies	1336
370. Serotonin: behavioral and physiological effects	1339
371. Biogenic amines and receptor regulation	1341
372. Cyclic nucleotides II	1346
373. Presynaptic mechanisms IV	1349
374. Action potentials and ion channels XI	1353
375. Action potentials and ion channels XII	1357
376. Basal ganglia II	1359
377. Glia IV	1364
378. Neuroendocrine controls: pituitary VII	1368
379. Characterization of muscarinic cholinergic receptors	1373
380. Neural control of immune system I	1378
381. Peptides: anatomical localization III	1383
382. Spinal cord II	1386
383. Regeneration: optic nerve	1389
384. Sprouting and sprouting mechanisms II	1391
385. Cell surface macromolecules I	1394
386. Photoreceptors	1397
387. Chemical sensory systems II	1399
388. Chemical sensory systems III	1404
389. Chemical sensory systems IV	1409
390. Brain metabolism III	1413
391. Specificity of synaptic connections I	1417
392. Specificity of synaptic connections II	1421
393. Synaptogenesis II	1423
394. Synaptogenesis III	1427

Session Number and Title — Page

Presidential Special Lecture—11:30 a.m.
395. Perspectives on Computational Neuroscience. T.J. Sejnowski ..... No abstract

Symposium—1:00 p.m.
396. Neuronal Serotonin Receptors. *Chaired by:* J.M. Palacios and B.P. Richardson 1430

Workshop—1:00 p.m.
397. New Directions in Mammalian CNS *in vitro:* Beyond the Slice. *Chaired by:* K.D. Walton.. 1430

Slide Sessions—1:00 p.m.
398. Human behavioral neurobiology III 1431
399. Subcortical visual pathways IV 1434
400. Neural control of immune system II 1437
401. Action potentials and ion channels XIII 1439
402. Peptides: physiological effects III 1443
403. Diseases of the nervous system: ischemia and Parkinson's disease 1446
404. Visual cortex VI 1449
405. Learning and memory: anatomy VI 1452
406. Specificity of synaptic connections and synaptogenesis 1455
407. Catecholamines V 1458
408. Aging and dementia: anatomy 1461
409. Cell lineage and differentiation V 1464
410. Auditory system IX 1467

Poster Sessions—1:00 p.m.
411. Neurotransmitters and receptors: histamine 1470
412. Transmitters and receptors in disease IV 1473
413. Process outgrowth V 1477
414. Process outgrowth VI 1482
415. Modulators III 1487
416. Nutritional and prenatal factors II 1491
417. Diseases of the nervous system: ischemic injury 1494
418. Diseases of the nervous system III 1499
419. Motor systems 1503
420. Structure and function of identified cells I 1510
421. Structure and function of identified cells II 1513
422. Structure and function of identified cells III 1516
423. Endocrine control of development II 1518
424. Peptide: receptors II 1522
425. Neuroendocrine controls: pituitary VIII 1527
426. Reflex function II 1531
427. Visual system: development and plasticity V 1534
428. Circuitry and pattern generation II 1539
429. Motivation and emotion II 1543
430. Stress, hormones and the autonomic nervous system II 1547
431. Excitotoxins II 1552
432. Excitatory amino acids: phencyclidine interaction 1554
433. N-methyl-D-aspartate: physiology 1557
434. Excitatory amino acids: localization and release 1562
435. Basal ganglia III 1566
436. Basal ganglia IV 1571

Session Number and Title — Page

437. Peptides: anatomical localization IV 1576
438. Neural control of immune system III 1581
439. Respiratory regulation I 1584
440. Pain modulation V 1588
441. Neural plasticity in adult animals III 1593
442. Developmental disorders 1598
443. Trophic agents I 1603
444. Trophic agents II 1608
445. Trophic interactions II 1612
446. Trophic interactions III 1616

Warner-Lambert Lecture—4:00 p.m.
447. The Acetylcholine Receptor: An Allosteric Protein Engaged in Intercellular Communication. J-P. Changeux ..... No abstract

SATURDAY

Symposia—8:30 a.m.
448. New Insights into the Structure and Function of Receptors. *Chaired by:* Z.W. Hall 1619
449. Hormonal Organization and Reorganization of Neural Circuits. *Chaired by:* A.P. Arnold 1619

Slide Sessions—8:30 a.m.
450. Neuroendocrine controls: other III 1620
451. Visual cortex VII 1623
452. Aging and dementia: function II 1626
453. Transmitters and receptors in disease V 1629
454. Brain metabolism IV 1632
455. Cell surface macromolecules II 1635
456. Respiratory regulation II 1638

Poster Sessions—8:30 a.m.
457. Catecholamines: metabolism and release 1641
458. Serotonin and biogenic amines 1644
459. Serotonin: electrophysiological studies 1648
460. Interactions between neurotransmitters IV 1653
461. Regional localization of receptors and transmitters IV 1657
462. Neural plasticity in adult animals IV 1660
463. Peptides: physiological effects IV 1666
464. Metabolism of transmitters and modulators 1671
465. Cellular aspects of disease I 1677
466. Neurotoxicity III 1681
467. Cellular aspects of disease II 1685
468. Visual system: development and plasticity VI ... 1689
469. Spinal cord and brainstem IV 1693
470. Disorders of motor systems and neural prostheses II 1698
471. Opiates, endorphins and enkephalins: anatomy and chemistry III 1701
472. Messenger RNA regulation V 1706
473. Membrane composition 1710
474. Behavioral pharmacology: acetylcholine 1713
475. Behavioral pharmacology: cocaine 1717
476. Behavioral pharmacology: addiction 1720

Thematic List of Sessions

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

Theme A: Development and Plasticity

Session Number	Session Title	Type	Day and Time
408.	Aging and dementia: anatomy	Slide	Fri PM
124.	Aging and dementia: function I	Poster	Wed AM
452.	Aging and dementia: function II	Slide	Sat AM
230.	Aging and dementia: molecular biology I	Slide	Thu AM
366.	Aging and dementia: molecular biology II	Poster	Fri AM
316.	Aging and dementia: plaques, tangles, amyloid	Poster	Thu PM
123.	Aging and dementia: transmitters	Poster	Wed AM
191.	Autonomic nervous system	Poster	Wed PM
257.	Biochemical and pharmacological correlates of development I	Poster	Thu AM
313.	Biochemical and pharmacological correlates of development II	Poster	Thu PM
339.	Biochemical and pharmacological correlates of development III	Slide	Fri AM
55.	Cell lineage and differentiation I	Slide	Tue PM
195.	Cell lineage and differentiation II	Poster	Wed PM
248.	Cell lineage and differentiation III	Poster	Thu AM
309.	Cell lineage and differentiation IV	Poster	Thu PM
409.	Cell lineage and differentiation V	Slide	Fri PM
200.	Development and plasticity: aging	Poster	Wed PM
168.	Development of invertebrates I	Slide	Wed PM
314.	Development of invertebrates II	Poster	Thu PM
442.	Developmental disorders	Poster	Fri PM
192.	Endocrine control of development I	Poster	Wed PM
423.	Endocrine control of development II	Poster	Fri PM
449.	**Hormonal Organization and Reorganization of Neural Circuits**	**Symp.**	**Sat AM**
315.	Limbic system	Poster	Thu PM
217.	**Molecular Genetic Analysis of Neuronal Development**	**Symp.**	**Thu AM**
74.	Morphogenesis and pattern formation I	Poster	Tue PM
308.	Morphogenesis and pattern formation II	Poster	Thu PM
419.	Motor systems	Poster	Fri PM
280.	Neural plasticity in adult animals I	Slide	Thu PM
341.	Neural plasticity in adult animals II	Slide	Fri AM
441.	Neural plasticity in adult animals III	Poster	Fri PM
462.	Neural plasticity in adult animals IV	Poster	Sat AM
269.	Neural plasticity in adult animals: hippocampus	Poster	Thu AM
47.	Neural plasticity in adult animals: spinal cord	Poster	Tue AM
7.	Neuronal death I	Slide	Tue AM
255.	Neuronal death II	Poster	Thu AM
220.	Neurotoxicity I	Slide	Thu AM
258.	Neurotoxicity II	Poster	Thu AM
466.	Neurotoxicity III	Poster	Sat AM
24.	Neurotoxicity: MPTP and ethanol	Poster	Tue AM
194.	Neurotoxicity: metals	Poster	Wed PM
193.	Nutritional and prenatal factors I	Poster	Wed PM
416.	Nutritional and prenatal factors II	Poster	Fri PM
6.	Process outgrowth I	Slide	Tue AM
75.	Process outgrowth II	Poster	Tue PM
130.	Process outgrowth III	Poster	Wed AM
337.	Process outgrowth IV	Slide	Fri AM
413.	Process outgrowth V	Poster	Fri PM
414.	Process outgrowth VI	Poster	Fri PM
104.	Process outgrowth: growth cone behavior	Slide	Wed AM
82.	Regeneration I	Poster	Tue PM
291.	Regeneration II	Slide	Thu PM
331.	Regeneration: PNS	Poster	Thu PM

268.	Regeneration: lower forms	Poster	Thu AM
383.	Regeneration: optic nerve	Poster	Fri AM
113.	Regeneration: spinal cord I	Slide	Wed AM
207.	Regeneration: spinal cord II	Poster	Wed PM
26.	Sensory systems: auditory, olfactory, gustatory	Poster	Tue AM
25.	Sensory systems: somatosensory	Poster	Tue AM
391.	Specificity of synaptic connections I	Poster	Fri AM
392.	Specificity of synaptic connections II	Poster	Fri AM
406.	Specificity of synaptic connections and synaptogenesis	Slide	Fri PM
48.	Sprouting and sprouting mechanisms I	Poster	Tue AM
384.	Sprouting and sprouting mechanisms II	Poster	Fri AM
106.	Synaptogenesis I	Slide	Wed AM
393.	Synaptogenesis II	Poster	Fri AM
394.	Synaptogenesis III	Poster	Fri AM
256.	Transmitter phenotypic plasticity	Poster	Thu AM
46.	Transplantation I	Poster	Tue AM
81.	Transplantation II	Poster	Tue PM
141.	Transplantation III	Poster	Wed AM
160.	Transplantation IV	Slide	Wed PM
219.	Transplantation for movement disorders	Slide	Thu AM
443.	Trophic agents I	Poster	Fri PM
444.	Trophic agents II	Poster	Fri PM
56.	Trophic agents: nerve growth factor I	Slide	Tue PM
142.	Trophic agents: nerve growth factor II	Poster	Wed AM
143.	Trophic agents: nerve growth factor III	Poster	Wed AM
152.	Trophic agents: nerve growth factor IV	Poster	Wed AM
282.	Trophic agents: nerve growth factor and others	Slide	Thu PM
162.	Trophic interactions I	Slide	Wed PM
445.	Trophic interactions II	Poster	Fri PM
446.	Trophic interactions III	Poster	Fri PM
71.	Visual system: development and plasticity I	Poster	Tue PM
167.	Visual system: development and plasticity II	Slide	Wed PM
285.	Visual system: development and plasticity III	Slide	Thu PM
344.	Visual system: development and plasticity IV	Slide	Fri AM
427.	Visual system: development and plasticity V	Poster	Fri PM
468.	Visual system: development and plasticity VI	Poster	Sat AM

Theme B: Cell Biology

Session Number	Session Title	Type	Day and Time
36.	Axonal and intracellular transport	Poster	Tue AM
52.	**The Basics of Molecular Biology**	**Symp.**	**Tue PM**
212.	Blood-brain barrier I	Poster	Wed PM
347.	Blood-brain barrier II	Slide	Fri AM
385.	Cell surface macromolecules I	Poster	Fri AM
455.	Cell surface macromolecules II	Slide	Sat AM
465.	Cellular aspects of disease I	Poster	Sat AM
467.	Cellular aspects of disease II	Poster	Sat AM
15.	Gene structure and function I	Slide	Tue AM
154.	Gene structure and function II	Poster	Wed AM
240.	Gene structure and function III	Poster	Thu AM
35.	Glia I	Poster	Tue AM
59.	Glia II	Slide	Tue PM
330.	Glia III	Poster	Thu PM
377.	Glia IV	Poster	Fri AM
473.	Membrane composition	Poster	Sat AM
107.	Messenger RNA regulation I	Slide	Wed AM
165.	Messenger RNA regulation II	Slide	Wed PM
302.	Messenger RNA regulation III	Poster	Thu PM

357.	Messenger RNA regulation IV	Poster	Fri AM
472.	Messenger RNA regulation V	Poster	Sat AM
182.	Metabolic studies	Poster	Wed PM
448.	**New Insights into the Structure and Function of Receptors**	**Symp.**	**Sat AM**
99.	**Proto-Oncogenes in the Nervous System**	**Symp.**	**Wed AM**
158.	**The Role of Nectins (Cell Binding Molecules) in Neural Development**	**Symp.**	**Wed PM**
189.	Staining and tracing techniques I	Poster	Wed PM
190.	Staining and tracing techniques II	Poster	Wed PM
420.	Structure and function of identified cells I	Poster	Fri PM
421.	Structure and function of identified cells II	Poster	Fri PM
422.	Structure and function of identified cells III	Poster	Fri PM

Theme C: Excitable Membranes and Synaptic Transmission

Session Number	Session Title	Type	Day and Time
29.	Action potentials and ion channels I	Poster	Tue AM
30.	Action potentials and ion channels II	Poster	Tue AM
31.	Action potentials and ion channels III	Poster	Tue AM
32.	Action potentials and ion channels IV	Poster	Tue AM
53.	Action potentials and ion channels V	Slide	Tue PM
147.	Action potentials and ion channels VI	Poster	Wed AM
148.	Action potentials and ion channels VII	Poster	Wed AM
163.	Action potentials and ion channels VIII	Slide	Wed PM
222.	Action potentials and ion channels IX	Slide	Thu AM
281.	Action potentials and ion channels X	Slide	Thu PM
374.	Action potentials and ion channels XI	Poster	Fri AM
375.	Action potentials and ion channels XII	Poster	Fri AM
401.	Action potentials and ion channels XIII	Slide	Fri PM
42.	Drug effects on receptors I	Poster	Tue AM
43.	Drug effects on receptors II	Poster	Tue AM
100.	**Peptide-Monoamine Interactions: From Molecular Mechanisms to Behavior**	**Symp.**	**Wed AM**
22.	Pharmacology of synaptic transmission I	Poster	Tue AM
23.	Pharmacology of synaptic transmission II	Poster	Tue AM
44.	Postsynaptic mechanisms I	Poster	Tue AM
45.	Postsynaptic mechanisms II	Poster	Tue AM
86.	Presynaptic mechanisms I	Poster	Tue PM
169.	Presynaptic mechanisms II	Slide	Wed PM
343.	Presynaptic mechanisms III	Slide	Fri AM
373.	Presynaptic mechanisms IV	Poster	Fri AM
87.	Synaptic structure and function I	Poster	Tue PM
88.	Synaptic structure and function II	Poster	Tue PM

Theme D: Neurotransmitters, Modulators, and Receptors

Session Number	Session Title	Type	Day and Time
327.	Acetylcholine: choline uptake	Poster	Thu PM
325.	Acetylcholine: localization	Poster	Thu PM
286.	Acetylcholine: metabolism I	Slide	Thu PM
326.	Acetylcholine: metabolism II	Poster	Thu PM
135.	Acetylcholine: muscarinic receptors	Poster	Wed AM
77.	Acetylcholine: regulation	Poster	Tue PM
249.	Adrenergic receptors	Poster	Thu AM
274.	Behavioral pharmacology I	Poster	Thu AM

289.	Behavioral pharmacology II	Slide	Thu PM
474.	Behavioral pharmacology: acetylcholine	Poster	Sat AM
476.	Behavioral pharmacology: addiction	Poster	Sat AM
475.	Behavioral pharmacology: cocaine	Poster	Sat AM
126.	Behavioral pharmacology: dopamine	Poster	Wed AM
170.	Behavioral pharmacology: dopamine and neuroleptics	Slide	Wed PM
371.	Biogenic amines and receptor regulation	Poster	Fri AM
251.	Biogenic amines: toxins	Poster	Thu AM
133.	Catecholamines I	Poster	Wed AM
134.	Catecholamines II	Poster	Wed AM
226.	Catecholamines III	Slide	Thu AM
253.	Catecholamines IV	Poster	Thu AM
407.	Catecholamines V	Slide	Fri PM
368.	Catecholamines: MPTP	Poster	Fri AM
369.	Catecholamines: anatomical studies	Poster	Fri AM
322.	Catecholamines: cell culture	Poster	Thu PM
252.	Catecholamines: electrophysiology	Poster	Thu AM
457.	Catecholamines: metabolism and release	Poster	Sat AM
197.	Characterization of cholinergic receptors I	Poster	Wed PM
223.	Characterization of cholinergic receptors II	Slide	Thu AM
379.	Characterization of muscarinic cholinergic receptors	Poster	Fri AM
260.	Characterization of neuronal nicotinic cholinergic receptors	Poster	Thu AM
2.	**Chemical Architecture of the Cerebellar Cortex: Structure and Function**	**Wksh.**	**Tue AM**
51.	**Cocaine: Modulation of Monoamine Function**	**Wksh.**	**Tue PM**
250.	Cyclic nucleotides I	Poster	Thu AM
372.	Cyclic nucleotides II	Poster	Fri AM
136.	Dopamine receptor functions	Poster	Wed AM
58.	Dopamine receptors	Slide	Tue PM
328.	Dopamine receptors: ligand binding and purification	Poster	Thu PM
434.	Excitatory amino acids: localization and release	Poster	Fri PM
208.	Excitatory amino acids: mechanisms	Poster	Wed PM
54.	Excitatory amino acids: pharmacology I	Slide	Tue PM
210.	Excitatory amino acids: pharmacology II	Poster	Wed PM
432.	Excitatory amino acids: phencyclidine interaction	Poster	Fri PM
109.	Excitatory amino acids: receptors I	Slide	Wed AM
209.	Excitatory amino acids: receptors II	Poster	Wed PM
287.	Excitotoxins I	Slide	Thu PM
431.	Excitotoxins II	Poster	Fri PM
267.	GABA and benzodiazepine receptors: behavioral studies	Poster	Thu AM
266.	GABA and benzodiazepine receptors: molecular characterization	Poster	Thu AM
263.	GABA and benzodiazepine: anatomy and cytochemistry	Poster	Thu AM
161.	GABA and benzodiazepine: pharmacology I	Slide	Wed PM
264.	GABA and benzodiazepine: pharmacology II	Poster	Thu AM
265.	GABA and benzodiazepine: receptor binding	Poster	Thu AM
340.	GABA and benzodiazepine: receptors	Slide	Fri AM
213.	Histochemical methods	Poster	Wed PM
196.	Interactions between neurotransmitters I	Poster	Wed PM
259.	Interactions between neurotransmitters II	Poster	Thu AM
354.	Interactions between neurotransmitters III	Poster	Fri AM
460.	Interactions between neurotransmitters IV	Poster	Sat AM
464.	Metabolism of transmitters and modulators	Poster	Sat AM
221.	Modulators I	Slide	Thu AM
312.	Modulators II	Poster	Thu PM
415.	Modulators III	Poster	Fri PM
433.	N-methyl-D-aspartate: physiology	Poster	Fri PM
396.	**Neuronal Serotonin Receptors**	**Symp.**	**Fri PM**
411.	Neurotransmitters and receptors: histamine	Poster	Fri PM
187.	Neurotransmitters: uptake, storage and secretion I	Poster	Wed PM
307.	Neurotransmitters: uptake, storage and secretion II	Poster	Thu PM
180.	Opiates, endorphins and enkephalins: anatomy and chemistry I	Poster	Wed PM
346.	Opiates, endorphins and enkephalins: anatomy and chemistry II	Slide	Fri AM

471.	Opiates, endorphins and enkephalins: anatomy and chemistry III	Poster	Sat AM
64.	Opiates, endorphins and enkephalins: physiological effects I	Slide	Tue PM
211.	Opiates, endorphins and enkephalins: physiological effects II	Poster	Wed PM
254.	Opiates, endorphins and enkephalins: physiological effects III	Poster	Thu AM
278.	Opiates, endorphins and enkephalins: physiological effects IV	Slide	Thu PM
361.	Opiates, endorphins and enkephalins: physiological effects V	Poster	Fri AM
284.	Peptide: receptors I	Slide	Thu PM
424.	Peptide: receptors II	Poster	Fri PM
83.	Peptides: anatomical localization I	Poster	Tue PM
273.	Peptides: anatomical localization II	Poster	Thu AM
381.	Peptides: anatomical localization III	Poster	Fri AM
437.	Peptides: anatomical localization IV	Poster	Fri PM
11.	Peptides: biosynthesis, metabolism and biochemical characterization I	Slide	Tue AM
299.	Peptides: biosynthesis, metabolism and biochemical characterization II	Poster	Thu PM
355.	Peptides: biosynthesis, metabolism and biochemical characterization III	Poster	Fri AM
39.	Peptides: opiate receptors	Poster	Tue AM
356.	Peptides: physiological effects I	Poster	Fri AM
362.	Peptides: physiological effects II	Poster	Fri AM
402.	Peptides: physiological effects III	Slide	Fri PM
463.	Peptides: physiological effects IV	Poster	Sat AM
155.	Peptides: receptors	Poster	Wed AM
118.	Peptides: substance P receptors	Poster	Wed AM
61.	Receptor regulation I	Slide	Tue PM
201.	Receptor regulation II	Poster	Wed PM
202.	Receptor regulation: cholinergic	Poster	Wed PM
324.	Receptor regulation: neuropeptides	Poster	Thu PM
198.	Regional localization of receptors and transmitters I	Poster	Wed PM
311.	Regional localization of receptors and transmitters II	Poster	Thu PM
358.	Regional localization of receptors and transmitters III	Poster	Fri AM
461.	Regional localization of receptors and transmitters IV	Poster	Sat AM
342.	Serotonin	Slide	Fri AM
458.	Serotonin and biogenic amines	Poster	Sat AM
323.	Serotonin receptors: radioligand binding studies	Poster	Thu PM
370.	Serotonin: behavioral and physiological effects	Poster	Fri AM
459.	Serotonin: electrophysiological studies	Poster	Sat AM
94.	Serotonin: functional studies I	Poster	Tue PM
224.	Serotonin: functional studies II	Slide	Thu AM
335.	**Stimulant-Induced Sensitization—Behavior and Pharmacology**	**Symp.**	**Fri AM**
66.	Transmitters and receptors in disease I	Slide	Tue PM
156.	Transmitters and receptors in disease II	Poster	Wed AM
300.	Transmitters and receptors in disease III	Poster	Thu PM
412.	Transmitters and receptors in disease IV	Poster	Fri PM
453.	Transmitters and receptors in disease V	Slide	Sat AM
16.	Transmitters in invertebrates I	Slide	Tue AM
70.	Transmitters in invertebrates II	Poster	Tue PM
298.	Transmitters in invertebrates III	Poster	Thu PM
349.	Transmitters in invertebrates IV	Slide	Fri AM

Theme E: Endocrine and Autonomic Regulation

Session Number	Session Title	Type	Day and Time
79.	Cardiovascular regulation: CNS pathways I	Poster	Tue PM
80.	Cardiovascular regulation: CNS pathways II	Poster	Tue PM
227.	Cardiovascular regulation: CNS pathways III	Slide	Thu AM
288.	Cardiovascular regulation: CNS pathways IV	Slide	Thu PM
345.	Cardiovascular regulation: CNS pathways V	Slide	Fri AM

205.	Cardiovascular regulation: heart, blood flow, nerves	Poster	Wed PM
206.	Cardiovascular regulation: hypertension	Poster	Wed PM
336.	**A Heart–Brain Peptide: The Atrial Natriuretic Factor**	**Symp.**	**Fri AM**
380.	Neural control of immune system I	Poster	Fri AM
400.	Neural control of immune system II	Slide	Fri PM
438.	Neural control of immune system III	Poster	Fri PM
115.	Neuroendocrine controls: other I	Poster	Wed AM
306.	Neuroendocrine controls: other II	Poster	Thu PM
450.	Neuroendocrine controls: other III	Slide	Sat AM
10.	Neuroendocrine controls: pituitary I	Slide	Tue AM
60.	Neuroendocrine controls: pituitary II	Slide	Tue PM
119.	Neuroendocrine controls: pituitary III	Poster	Wed AM
186.	Neuroendocrine controls: pituitary IV	Poster	Wed PM
233.	Neuroendocrine controls: pituitary V	Poster	Thu AM
301.	Neuroendocrine controls: pituitary VI	Poster	Thu PM
378.	Neuroendocrine controls: pituitary VII	Poster	Fri AM
425.	Neuroendocrine controls: pituitary VIII	Poster	Fri PM
276.	**Neuropeptides/Neural Systems in Fever, Inflammation and Immune Responses**	**Symp.**	**Thu PM**
78.	Regulation of autonomic function I	Poster	Tue PM
110.	Regulation of autonomic function II	Slide	Wed AM
203.	Regulation of autonomic function III	Poster	Wed PM
204.	Regulation of autonomic function IV	Poster	Wed PM
439.	Respiratory regulation I	Poster	Fri PM
456.	Respiratory regulation II	Slide	Sat AM
4.	**Visceral Afferents: Signaling and Integration**	**Wksh.**	**Tue AM**

Theme F: Sensory Systems

Session Number	Session Title	Type	Day and Time
17.	Auditory system I	Slide	Tue AM
89.	Auditory system II	Poster	Tue PM
90.	Auditory system III	Poster	Tue PM
91.	Auditory system IV	Poster	Tue PM
149.	Auditory system V	Poster	Wed AM
150.	Auditory system VI	Poster	Wed AM
151.	Auditory system VII	Poster	Wed AM
350.	Auditory system VIII	Slide	Fri AM
410.	Auditory system IX	Slide	Fri PM
102.	Chemical sensory systems I	Slide	Wed AM
387.	Chemical sensory systems II	Poster	Fri AM
388.	Chemical sensory systems III	Poster	Fri AM
389.	Chemical sensory systems IV	Poster	Fri AM
218.	**Information Processing in the Macaque Retina**	**Wksh.**	**Thu AM**
40.	Invertebrate sensory processing I	Poster	Tue AM
41.	Invertebrate sensory processing II	Poster	Tue AM
33.	Pain modulation I	Poster	Tue AM
84.	Pain modulation II	Poster	Tue PM
272.	Pain modulation III	Poster	Thu AM
283.	Pain modulation IV	Slide	Thu PM
440.	Pain modulation V	Poster	Fri PM
34.	Pain: central pathways I	Poster	Tue AM
164.	Pain: central pathways II	Slide	Wed PM
329.	Pain: central pathways IV	Poster	Thu PM
386.	Photoreceptors	Poster	Fri AM
12.	Retina I	Slide	Tue AM
108.	Retina II	Slide	Wed AM
293.	Retina III	Poster	Thu PM
294.	Retina IV	Poster	Thu PM

360.	Retina V	Poster	Fri AM
57.	Somatic afferents I	Slide	Tue PM
214.	Somatic afferents II	Poster	Wed PM
73.	Somatosensory cortex and thalamocortical relationships I	Poster	Tue PM
131.	Somatosensory cortex and thalamocortical relationships II	Poster	Wed AM
132.	Somatosensory cortex and thalamocortical relationships III	Poster	Wed AM
144.	Spinal cord I	Poster	Wed AM
382.	Spinal cord II	Poster	Fri AM
271.	Subcortical somatosensory pathways	Poster	Thu AM
145.	Subcortical somatosensory pathways: trigeminal	Poster	Wed AM
62.	Subcortical visual pathways I	Slide	Tue PM
122.	Subcortical visual pathways II	Poster	Wed AM
241.	Subcortical visual pathways III	Poster	Thu AM
399.	Subcortical visual pathways IV	Slide	Fri PM
5.	Visual cortex I	Slide	Tue AM
101.	Visual cortex II	Slide	Wed AM
177.	Visual cortex III	Poster	Wed PM
178.	Visual cortex IV	Poster	Wed PM
292.	Visual cortex V	Poster	Thu PM
404.	Visual cortex VI	Slide	Fri PM
451.	Visual cortex VII	Slide	Sat AM

Theme G: Motor Systems and Sensorimotor Integration

Session Number	Session Title	Type	Day and Time
13.	Basal ganglia I	Slide	Tue AM
376.	Basal ganglia II	Poster	Fri AM
435.	Basal ganglia III	Poster	Fri PM
436.	Basal ganglia IV	Poster	Fri PM
270.	Basal ganglia: electrophysiology and behavior	Poster	Thu AM
231.	Circuitry and pattern generation I	Slide	Thu AM
428.	Circuitry and pattern generation II	Poster	Fri PM
8.	Control of posture and movement I	Slide	Tue AM
95.	Control of posture and movement II	Poster	Tue PM
96.	Control of posture and movement III	Poster	Tue PM
105.	Control of posture and movement IV	Slide	Wed AM
199.	Control of posture and movement V	Poster	Wed PM
244.	Control of posture and movement VI	Poster	Thu AM
321.	Control of posture and movement VII	Poster	Thu PM
72.	Cortex: motor cortex I	Poster	Tue PM
188.	Cortex: motor cortex II	Poster	Wed PM
304.	Cortex: prefrontal and premotor areas	Poster	Thu PM
117.	Disorders of motor systems and neural prostheses I	Poster	Wed AM
470.	Disorders of motor systems and neural prostheses II	Poster	Sat AM
295.	Invertebrate motor function	Poster	Thu PM
69.	Motor systems and sensorimotor integration: cerebellum I	Poster	Tue PM
171.	Motor systems and sensorimotor integration: cerebellum II	Slide	Wed PM
351.	Motor systems and sensorimotor integration: cerebellum III	Poster	Fri AM
49.	Motor systems and sensorimotor integration: oculomotor system I	Poster	Tue AM
112.	Motor systems and sensorimotor integration: oculomotor system II	Slide	Wed AM
303.	Motor systems and sensorimotor integration: oculomotor system III	Poster	Thu PM
179.	Motor systems and sensorimotor integration: vestibular system I	Poster	Wed PM
338.	Motor systems and sensorimotor integration: vestibular system II	Slide	Fri AM

363.	Motor systems and sensorimotor integration: vestibular system III	Poster	Fri AM
332.	Muscle I	Poster	Thu PM
333.	Muscle II	Poster	Thu PM
296.	Reflex function I	Poster	Thu PM
426.	Reflex function II	Poster	Fri PM
21.	Spinal cord and brainstem I	Poster	Tue AM
232.	Spinal cord and brainstem II	Slide	Thu AM
239.	Spinal cord and brainstem III	Poster	Thu AM
469.	Spinal cord and brainstem IV	Poster	Sat AM

Theme H: Structure and Function of the CNS

Session Number	Session Title	Type	Day and Time
247.	Amygdala and limbic cortex	Poster	Thu AM
125.	Basal forebrain and cholinergic pathways	Poster	Wed AM
37.	Brain metabolism I	Poster	Tue AM
228.	Brain metabolism II	Slide	Thu AM
390.	Brain metabolism III	Poster	Fri AM
454.	Brain metabolism IV	Slide	Sat AM
352.	Clinical CNS neurophysiology I	Poster	Fri AM
353.	Clinical CNS neurophysiology II	Poster	Fri AM
85.	Comparative neuroanatomy: amphibians, reptiles and birds	Poster	Tue PM
38.	Comparative neuroanatomy: fish and below	Poster	Tue AM
146.	Comparative neuroanatomy: mammals, etc.	Poster	Wed AM
137.	Diseases of the nervous system I	Poster	Wed AM
138.	Diseases of the nervous system II	Poster	Wed AM
418.	Diseases of the nervous system III	Poster	Fri PM
403.	Diseases of the nervous system: ischemia and Parkinson's disease	Slide	Fri PM
417.	Diseases of the nervous system: ischemic injury	Poster	Fri PM
348.	Diseases of the nervous system: viral and traumatic injury to CNS	Slide	Fri AM
103.	Epilepsy I	Slide	Wed AM
318.	Epilepsy II	Poster	Thu PM
317.	Epilepsy: brain slices	Poster	Thu PM
261.	Epilepsy: human and genetic models	Poster	Thu AM
262.	Epilepsy: kindling	Poster	Thu AM
367.	Hippocampus	Poster	Fri AM
319.	Hypothalamus	Poster	Thu PM
397.	**New Directions in Mammalian CNS *in vitro:* Beyond the Slice**	**Wksh.**	**Fri PM**
159.	**Structural Computer Simulations in Developmental and Systems Neurobiology**	**Symp.**	**Wed PM**

Theme I: Neural Basis of Behavior

Session Number	Session Title	Type	Day and Time
93.	Alcohol and barbiturates I	Poster	Tue PM
139.	Alcohol and barbiturates II	Poster	Wed AM
140.	Alcohol and barbiturates III	Poster	Wed AM
19.	Biological rhythms I	Poster	Tue AM
65.	Biological rhythms II	Slide	Tue PM
120.	Biological rhythms III	Poster	Wed AM
172.	Biological rhythms IV	Slide	Wed PM
242.	Biological rhythms V	Poster	Thu AM
290.	Biological rhythms VI	Slide	Thu PM

27.	Chronic drugs and neurotoxicity I	Poster	Tue AM
28.	Chronic drugs and neurotoxicity II	Poster	Tue AM
9.	Feeding and drinking I	Slide	Tue AM
92.	Feeding and drinking II	Poster	Tue PM
129.	Feeding and drinking III	Poster	Wed AM
153.	Feeding and drinking IV	Poster	Wed AM
166.	Feeding and drinking V	Slide	Wed PM
245.	Feeding and drinking VI	Poster	Thu AM
320.	Feeding and drinking VII	Poster	Thu PM
20.	Hormonal control of behavior I	Poster	Tue AM
68.	Hormonal control of behavior II	Poster	Tue PM
183.	Human behavioral neurobiology I	Poster	Wed PM
238.	Human behavioral neurobiology II	Poster	Thu AM
398.	Human behavioral neurobiology III	Slide	Fri PM
18.	Interhemispheric relations	Poster	Tue AM
111.	Invertebrate learning and behavior I	Slide	Wed AM
175.	Invertebrate learning and behavior II	Poster	Wed PM
229.	Invertebrate learning and behavior III	Slide	Thu AM
63.	Learning and memory: anatomy I	Slide	Tue PM
181.	Learning and memory: anatomy II	Poster	Wed PM
225.	Learning and memory: anatomy III	Slide	Thu AM
297.	Learning and memory: anatomy IV	Poster	Thu PM
310.	Learning and memory: anatomy V	Poster	Thu PM
405.	Learning and memory: anatomy VI	Slide	Fri PM
184.	Learning and memory: pharmacology I	Poster	Wed PM
235.	Learning and memory: pharmacology II	Poster	Thu AM
237.	Learning and memory: pharmacology III	Poster	Thu AM
173.	Learning and memory: physiology I	Slide	Wed PM
236.	Learning and memory: physiology II	Poster	Thu AM
305.	Learning and memory: physiology III	Poster	Thu PM
364.	Learning and memory: physiology IV	Poster	Fri AM
14.	Monoamines and behavior I	Slide	Tue AM
67.	Monoamines and behavior II	Poster	Tue PM
116.	Monoamines and behavior III	Poster	Wed AM
185.	Monoamines and behavior IV	Poster	Wed PM
234.	Monoamines and behavior V	Poster	Thu AM
365.	Motivation and emotion I	Poster	Fri AM
429.	Motivation and emotion II	Poster	Fri PM
3.	**A Neural Systems Approach to the Analysis of Fear and Anxiety**	**Symp.**	**Tue AM**
114.	Neuroethology I	Slide	Wed AM
176.	Neuroethology II	Poster	Wed PM
243.	Neuroethology III	Poster	Thu AM
121.	Neuropeptides and behavior I	Poster	Wed AM
174.	Neuropeptides and behavior II	Poster	Wed PM
246.	Psychotherapeutic drugs: antidepressants	Poster	Thu AM
128.	Psychotherapeutic drugs: antipsychotics	Poster	Wed AM
127.	Psychotherapeutic drugs: anxiolytics	Poster	Wed AM
76.	Sleep	Poster	Tue PM
359.	Stress, hormones and the autonomic nervous system I	Poster	Fri AM
430.	Stress, hormones and the autonomic nervous system II	Poster	Fri PM

416.9 THE LATER BEHAVIOUR OF UNDERNOURISHED, ARTIFICIALLY-REARED RATS. J.L.Smart*, A.E.Billing*, J.P.Duggan* and R.F.Massey* (SPON: European Neuroscience Association). Dept. of Child Health, University of Manchester, Medical School, Oxford Road, Manchester M13 9PT, England.

Artificial rearing was employed to obviate the possibility of maternal mediation of effects. Four groups of rat pups were reared: mother-reared control (well-fed) and undernourished (MRC, MRU) and artificially-reared control and undernourished (ARC, ARU). Pups for artificial rearing were fitted with a gastric cannula on postnatal day 5 and were fed by intermittent gastric infusion expressed rats' milk (days 5-7), mixtures of rats' milk and milk substitute (days 8-16), and milk substitute only (days 17-20). Undernutrition, imposed from 5-25 postnatal days, was effected initially by underfeeding the mother (MRU) or by infusing restricted quantities of milk (ARU). Weaning was at 21 days and undernutrition from 21-25 days was by restricting the supply of solid food. All rats were fed ad lib from 25 days. There were a total of 48 male rats: 13 MRC, 13 MRU, 12 ARC and 10 ARU and, within each group, all rats were drawn from different litters. Rats were subjected to three tests of behaviour starting at 18 weeks.

Spontaneous alternation was tested, two trials a day with a 30 s inter-trial interval, on three consecutive days. There were no significant effects of treatment on days 1 and 3, but there was on day 2 ($p<.05$) due to the low alternation rate of the ARU rats (30%).

Conditioned food preferences were tested by the method of Booth, D.A. (Physiol. Psychol., 2: 344, 1974). All groups showed a significant preference for carbohydrate- over non-nutritive-paired odours, and all except the ARC group showed a preference for protein over non-nutritive; but, whereas both U groups showed significant preferences for carbohydrate- over protein-paired odours, the MRC group showed no preference and the ARC group a preference for the protein. For the carbohydrate vs protein test there were significant effects of nutrition ($p<.01$) but not of rearing.

Visual discrimination (circle vs triangle) was tested using a modified Lashley jumping stand method (Fleischer, S.F. and Turkewitz, G., Devl Psychobiol., 12: 137, 1979). Rats were given 23 daily training sessions, each comprising 10 trials. Only 6 rats learned the discrimination, possibly because the distance to be jumped was too great (mean 17 cm). However, there were highly significant effects of nutrition, but not of rearing, on latency to jump ($p<.001$). U rats displayed longer latencies than C rats.

Hence for two aspects of behaviour the effects of early undernutrition were the same in both MR and AR rats and cannot have been mediated through alterations in maternal care.

416.10 EFFECTS OF EARLY MALNUTRITION ON LIGHT-DEPENDENT CHANGES IN RAT PINEAL GLAND BIOCHEMISTRY. L.D. Lytle, K.A. Haak*, and G. Torres* (SPON: G.H. Jacobs). Department of Psychology, University of California, Santa Barbara, CA 93106.

Impaired physiology and behavior caused by early malnutrition may result from temporary or permanent impairments in synaptic function, but the lack of an appropriate animal model has made it difficult to precisely characterize these impairments. In the present studies we attempted to localize malnutrition-induced alterations in an oligosynaptic nerve circuit that normally mediates light-induced changes in pineal gland synthesis of the endocrine hormone melatonin.

Melatonin production depends in part on changes in N-acetyltransferase (NAT), an enzyme whose activity is maximal at night and lowest during the day. Brief nocturnal light exposure rapidly suppresses adult rat NAT activity via a retina:brain:spinal cord:sympathetic nervous system circuit (Klein and Weller, 1972). Nocturnal light-induced suppression of NAT activity cannot be detected until 6 days postnatal, when retinal and/or neural mechanisms sensitive to light first begin to function (Bronstein et al., 1985). Early malnutrition delays the maturation of this response by 3-4 days (Haak et al., 1986).

In the present experiments we used drugs with specific effects on noradrenergic transmission as probes to identify some of the synaptic sites that might be affected by early malnutrition. Pineal glands were obtained from 7 day-old albino rats whose mothers were fed isocaloric adequate (24%) or low (8%) protein diets from the time of conception. Pineal glands removed during the daytime were incubated in vitro with the indirect acting sympathomimetic drug, d-amphetamine sulfate, to assess possible malnutrition-induced changes in postganglionic sympathetic noradrenergic nerve function. Changes in NAT activity were measured 4 hr later, using the method of Deguchi and Axelrod (1972). Amphetamine induced elevations in pineal gland NAT activity were similar in both groups. Although early malnutrition may have few effects on the functioning of sympathetic noradrenergic synapses that control NAT activity, it now seems likely that it could delay the maturation of retina and/or brain mechanisms important for mediating light-induced changes in pineal gland hormone biochemistry in developing animals.

416.11 DAILY PRENATAL STRESS DURING THE LAST THIRD OF GESTATION DECREASES VULNERABILITY TO STRESS IN THE RAT OFFSPRING. E. Fride*, B.Z. Roitjberg*, M. Sadeh* and M. Weinstock*. (SPON: European Neuroscience Association). Dept. Pharmacology, Hebrew University of Jerusalem, Israel.

Previous experiments have shown that the outcome of maternal noise and light stress on early behavioral development, depends on the timing and pattern of the stress stimulus(1). This may also determine the behavioral pattern of the offspring at adulthood in response to stressful situations. We have already shown that stress sessions randomly applied (3/week; R)throughout pregnancy, increased fearfullness and reactivity to anxiety-provoking situations(2,3). In the present experiments, we studied the influence of daily stress during the last week of gestation (DL) on the response of the adult offspring to a variety of stressful situations.

Pregnant rats were exposed to daily four hour sessions of noise (95 dB SPL) and flashing lights (20 fc) during the last week of gestation. The response of the adult offspring to a novel environment was assessed by measuring the increase in plasma corticosterone (CCS) and number of fecal pellets voided, on exposure to an open-field. The level of timidity was assessed from the time spent on the open arms of a "plus maze". Pup retrieval by the adult female offspring was also determined under normal and conflict conditions.

It was found that DL ♂ and ♀ offspring voided significantly fewer fecal pellets and secreted lower levels of CCS into the bloodstream on exposure to the open-field than controls ($p<0.05$). DL offspring spent more time on the open arms of the "plus maze" than controls ($p<0.05$). Pup retrieval under conflict conditions was not disrupted more than in controls.

We conclude that stress applied daily during the last week of gestation, decreases timidity and reactivity of the offspring to novel or stressful environments. This is in direct contrast to the outcome of stress applied randomly throughout pregnancy.

1. Fride, E. and M. Weinstock. Devl. Psychobiol. 17: 651, 1984.
2. Fride, E., Y. Dan, M. Gavish and M. Weinstock. Life Sciences 36: 2103, 1985.
3. Fride, E., Y. Dan, J. Feldon, G. Halevy and M. Weinstock. Physiol. Behav. 37: 681, 1986.

416.12 EARLY DEVELOPMENT OF RATS DELIVERED AT PRETERM GESTATIONAL AGES. C. R. Almli, D. Rubenstein* and J. Miller*. Programs in Neural Science and Occupational Therapy, Departments of Anatomy and Neurobiology, and Psychology, Wash. Univ. Sch. Med., St. Louis, Mo. 63110.

The development of fetal "behavior" is being heavily investigated in humans and other animals. In humans, fetuses are being studied in utero (ultrasonography), while rat research externalizes the fetus with maintenance of placental attachment to the mother. This research is showing the relatively vast behavioral complexity displayed by even very young fetuses in terms of "spontaneous" and "stimulation-induced" movement patterns. In the present investigation, fetal rats were completely delivered from their mothers (i.e., premature delivery) and their "spontaneous" behaviors were studied over an 8 hour period.

Timed-pregnant, female albino rats were killed (cervical fracture) and a laparotomy was performed to deliver the premature pups at 18.5, 19.5 and 20.5 days estimated gestational age (EGA). The pups were quickly freed of the uterus and amnion, and gently brushed to stimulate breathing. Vaginally delivered pups (21.5 EGA), and preterm pups, were incubated at 32-35° C. One hour after the onset of regular respiration, pups were studied for "spontaneous" movement (head, mouth, forelimbs, hindlimbs, twitch, curl and stretch). Movement studies were repeated 7 times over 7 hours.

Mortality rates of premature pups ranged from 100% at EGA-18.5 days (all pups died by 4.2 hours post-delivery), to 3% at EGA-20.5 days. Body weight and length, brain weight, and cerebral width and length increased with age. Pups from 19.5 to 21.5 days EGA were similar for movement patterns. Pups at 18.5 days EGA differed from the others (e.g., lower total movement, lower percentage of hindlimb movements).

These results show that, with proper care, rat pups can be delivered prematurely and studied behaviorally for up to 8 hours. Movements of "premature" pups compare favorably with "externalized" rat fetuses when similar measures are used. The goals of this research program are to (1) study developmental transitions from prenatal to postnatal life, and (2) develop an "animal model" of human premature infants.

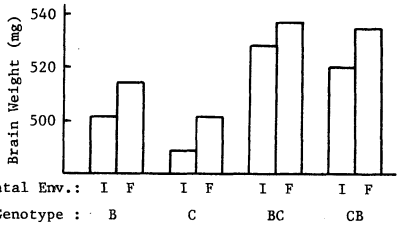
416.13 EFFECTS OF SLEEP/WAKING STATES ON RECURRENT INHIBITION IN DENTATE GYRUS. K. Austin, J. Bronzino and P.J. Morgane (SPON: J. McKearney). Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 01545 and Department of Engineering, Trinity College, Hartford, Conn. 06106.

We previously showed that prenatal protein malnutrition affects long-term plasticity (long-term potentiation) in the dentate gyrus (Dev. Brain Res. 29:267-273, 1986). There is also evidence that transmission through the hippocampal trisynaptic circuit is vigilance state dependent (J. Neurophysiol. 41:716-732, 1978). The possibility that both malnutrition and vigilance state may influence activity in hippocampal circuits by modification of recurrent inhibitory processes is currently under investigation. In the present studies we examined the effects of sleep/waking states on recurrent inhibition in the dentate gyrus in order to determine the role of inhibitory interneurons in the vigilance state modulation of evoked extracellular field potentials. Paired-pulse tests were carried out during each vigilance state (active waking, immobile waking, slow-wave sleep and REM sleep). The paired pulse technique is a useful tool which allows us to monitor the degree of recurrent inhibition in effect in the dentate gyrus. We first determined the stimulus-response relationship and found that a population spike 90% of maximum could be obtained using a stimulus of 600 μA by 100 μsec. Using this intensity, paired pulse tests were performed during each vigilance state using interpulse intervals of 30 and 40 msec. Population spike amplitudes from the first evoked waveform were grouped according to vigilance state and plotted and analyzed using the Bonferroni T-test. The population spike amplitudes during immobile waking were found to be significantly smaller than during the other vigilance states tested. This is in line with earlier findings of Winson and Abzug (1978) who showed that population spikes in the dentate gyrus are larger during slow-wave sleep than during immobile waking. Using paired pulse procedures we then showed that recurrent inhibition is itself modified as a function of vigilance state. We found that recurrent inhibitory influences are significantly more effective in inhibiting the second spike 30 msec later during slow-wave sleep and immobile waking than during REM sleep and active waking. This data indicates that recurrent inhibitory processes in the dentate gyrus are vigilance state dependent and suggests that local circuit interneurons play an important role in short-term, vigilance state modulated plasticity. Experiments in progress seek to further establish how recurrent inhibition changes as a function of vigilance states and whether these short-term plastic responses are also altered by prenatal protein malnutrition. Changes in the efficacy of neuronal transmission at different loci along the trisynaptic circuit are also being examined. (Supported by NIH grants HD-06364-13 and HD-23338-01).

416.14 HYBRID VIGOR AND MATERNAL ENVIRONMENT EFFECTS ON MOUSE BRAIN SIZE. B. Bulman-Fleming*, D. Wahlstèn, and J.M. Lassalle*. (SPON: R. Beauchamp) Dept. of Psychology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1 (D.W. and B. B-F) and Lab. d'Ethologie et de Psychophysiologie, Université de Tours, 37200 Tours, France (J.M.L.)

A combination of ovarian grafting and surrogate fostering was employed with BALB/cWah (B), C57BL/6J (C), and their reciprocal F_1 hybrids (BC and CB) such that each of these 4 genotypes experienced a grafted prenatal environment (I or F) and was fostered at birth to an ungrafted lactating dam (I or F). Weaning was at 21 days and perfusion and brain extraction at 100 days of age. The 16 combinations of genotype (G), prenatal (E), and postnatal (P) maternal environments resulted in 355 and 337 mice at birth and 100 days, respectively.

After adjusting for litter size effects, the 100 day brain weights were about 12mg heavier for strain B than C and about 29mg heavier for the hybrids than for the average of the inbreds. Animals which experienced the hybrid (F) postnatal maternal environment had brains which were about 12mg heavier than those which experienced the inbred (I) postnatal environment (see diagram). There were no prenatal maternal environment effects on adult brain weight.



At birth, body weights of F_1 pups were significantly heavier (about .19g) than those of inbred pups. Pups which experienced an F prenatal environment were significantly heavier than those experiencing an I environment and this effect was larger for inbred than for hybrid pups (.10g and .05g), respectively). By 100 days of age the prenatal effect on body weight was no longer evident. The pattern of results for adult body weight was very similar to that described for brain weight, except that there was a large sex difference in body weight.

In general, effects of maternal environment are similar in magnitude to effects produced by genetic differences.

DISEASES OF THE NERVOUS SYSTEM: ISCHEMIC INJURY

417.1 **ATTENUATION OF POST-HEMORRHAGIC AND POST-ISCHEMIC CEREBRAL HYPOPERFUSION BY THE 21-AMINOSTEROID U-74006F, A POTENT INHIBITOR OF IRON-DEPENDENT LIPID PEROXIDATION.** M.A.Travis, P.A.Yonkers, and E.D.Hall, CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

The ability of the 21-aminosteroid U-74006F (21-(4-(2,6,di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl)-16 -methyl-pregna-1,4,9(11)-triene-3,20-dione, monomethane sulfonate), a potent inhibitor of iron-dependent lipid peroxidation, to antagonize the phenomenon of progressive brain hypoperfusion following either subarachnoid hemorrhage (SAH) or a brief (5-minute) episode of global brain ischemia was examined in anesthetized cats. SAH was experimentally produced by injection of 0.5 ml/kg of unheparinized autologous blood into the cisterna magna after prior withdrawal of an equivalent volume of cerebrospinal fluid. In animals that received an i.v. dose of vehicle at 30 minutes post-SAH, there was a progressive decline in caudate nuclear blood flow (CNBF; -50.6% by 3 hours) and increase in intra-cerebral pressure (ICP; +18.5 mmHg by 3 hours). In comparison, in cats that received a 1 mg/kg i.v. dose of U-74006F at 30 minutes after SAH, there was a complete prevention of the fall in CNBF and a significant attenuation of the rise in ICP. Furthermore, the drug reduced a concomitant fall in the mean arterial blood pressure and cerebral perfusion pressure. While not as effective as the 1 mg/kg dose, a 0.1 mg/kg dose also significantly attenuated the post-SAH fall in CNBF.

In a second set of experiments, cortical blood flow (CBF) returned to normal or above immediately following a 5 minute period of near total brain ischemia (tourniquet-induced). Thereafter, CBF fell progressively to a level 71.7% below normal by 3 hours post-ischemia. In contrast, in cats that received a 1 mg/kg i.v. dose of U-74006F at 15 minutes after the ischemia episode, CBF remained significantly above that seen in the vehicle-treated animals. At 3 hours, CBF had declined by only 45.7% ($p<0.04$ compared to vehicle treatment). In addition, U-74006F treatment significantly improved post-ischemic maintenance of blood pressure, recovery of the somatosensory evoked potential, and reduced post-ischemic arterial blood acidosis.

These results support the view that microvascular lipid peroxidation is involved in both post-ischemic and post-hemorrhagic brain hypoperfusion and suggest that the 21-aminosteroid U-74006F may be useful in the early treatment of ischemic or hemorrhagic stroke.

417.2 PROTECTION AGAINST POST-ISCHEMIC MORTALITY AND NEURONAL NECROSIS IN GERBILS BY THE 21-AMINOSTEROID U74006F, A POTENT INHIBITOR OF LIPID PEROXIDATION. K.P.Berry*, J.M.Braughler and E.D.Hall. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001

The possible protective effects of U74006F, a novel 21-aminosteroid (21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16α-methyl-pregna-1,4,9(11)-triene-3,20-dione, monomethane sulfonate) inhibitor of CNS tissue lipid peroxidation that is devoid of classical steroid hormonal activities, was examined on post-ischemic mortality and neuronal necrosis in gerbil brain following a 3 hr. period of unilateral carotid occlusion. Male Mongolian gerbils (55-65 g) received two i.p. injections of vehicle or U74006F (3 or 10 mg/kg). The first injection was given 10 min. before carotid occlusion and the second was administered at the end of the 3 hr. carotid occlusion. Only those gerbils showing definite clinical signs of brain ischemia by 1 hr. after occlusion were included. All experiments were carried out blind.

In a first series of experiments, vehicle treated gerbils displayed a 60.9% survival at 24 hrs. post-ischemia which dropped to 34.8% at 48 hrs. In contrast, the 10 mg/kg U74006F treated group showed an 86.7% survival at 24 hrs. ($p<0.1$ vs vehicle) and an 80.0% survival at 48 hrs. ($p<0.01$). The 3 mg/kg treated animals displayed only a slight improvement in survival at 24 hrs. at 78.6%, but no difference from vehicle by 48 hrs. at 28.6%.

In a second series, a comparison of neuronal densities was made in the hippocampal CA_1 subfield and medial and lateral cerebral cortex in surviving animals at 24 hrs. after unilateral carotid occlusion. Comparing the cell densities in the ischemic hemisphere to those in the contralateral non-ischemic hemisphere revealed a significantly better neuronal preservation as a result of U74006F (10 mg/kg i.p. x 2) treatment. In the vehicle animals, the mean neuronal densities, in relation to the non-ischemic hemisphere, were 13.5% in the CA_1 subfield, 70.4% in the medial cortex, and 9.1% in the lateral cortex. In U74006F treated gerbils, the mean densities were 52.0% in the CA_1 subfield ($p<0.003$ vs vehicle), 90.1% in the medial cortex ($p<0.02$) and 49.8% in the lateral cortex ($p<0.002$).

These results show that U74006F can improve survival and attenuate neuronal necrosis in a severe brain ischemia model and provide pharmacological support for an important role of oxygen radical-induced LP in the pathophysiology of brain ischemia followed by reperfusion.

417.3 **U-74006F, AN INHIBITOR OF LIPID PEROXIDATION, PROTECTS AGAINST LESION DEVELOPMENT FOLLOWING EXPERIMENTAL STROKE IN THE CAT: HISTOLOGICAL AND METABOLIC ANALYSIS.** R.C.Silvia, M.F.Piercey, W.E.Hoffmann*, R.L.Chase, J.M.Braughler and A.H.Tang, (Spon. L.Williams), CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

One mechanism theorized to underlie the degenerative sequelae to a major stroke is the appearance of oxidative free radicals accompanying aberrant cellular metabolism. Free radicals may irreparably damage cell membranes via lipid peroxidation (LP). To evaluate the role of LP in stroke pathophysiology, we have examined the effects of treating experimental stroke with U-74006F (21-(4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl)-16a-methyl-pregna-1,4,9(11)-triene-3,20-dione, monomethane sulfonate). In various in vitro assays using brain homogenates, U-74006F has been found to be a potent and effective inhibitor of iron-dependent LP. With a 50% inhibitory concentration of 18 uM, the potency of U-74006F approaches that of a-tocopherol. In contrast to a-tocopherol, however, U-74006F may be given intravenously and is rapidly taken up by neural tissue. One-hour unilateral occlusion of the middle cerebral artery in the cat resulted in massive ipsilateral lesions measured one week later by classical histology and quantitative 2-deoxyglucose autoradiography. Lesions were characterized by large infarct areas of reduced metabolic rates surrounded by a penumbra of enhanced metabolic rates. Spectral analysis revealed that lesion sides had increased numbers of pixels with metabolic rates below 11 or above 30 umole glucose/100g brain/min. Post-occlusion treatment of cats with multiple i.v. injections of U-74006F (1 mg/kg 15 min after reflow, and 0.5 mg/kg 2, 6, and 12 hr later) resulted in dramatic reductions in lesion sizes, mainly through reductions in metabolically depressed infarct areas. Highly significant reductions were noted in lesion volume measured by spectral analysis or by tracing lesion areas. Lesion area in sections showing maximal metabolic damage were reduced by 76% in the cortex and 64% in the caudate. Lesion volumes were estimated to be reduced by 81% in both cortex and caudate. It is concluded that (1) LP may represent a critical early post-ischemic step underlying stroke pathophysiology, and (2) U-74006F may represent a new and significant therapeutic entity for treatment of this debilitating condition.

417.4 EFFECTS OF HYPOXIA ON RESPONSES TO ACIDIC AMINO ACIDS IN THE IN VITRO HIPPOCAMPAL SLICE. R.K. Rader*, T.H. Lanthorn, P.S. Lipton[1]. Searle Research & Development, Chesterfield, Mo. 63198; [1]Dept. of Physiology, Univ. of Wisconsin, Madison, WI. 53706.

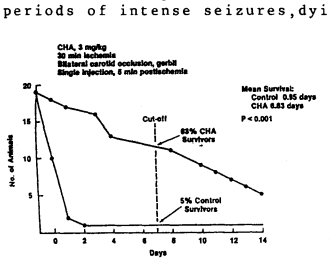
Hypoxia can produce neuronal damage in vivo and in vitro. During hypoxia in the in vitro hippocampal slice, synaptic responses are abolished. This could be due to presynaptic or postsynaptic factors. In order to determine the relative importance of pre- and post-synaptic mechanisms we have recorded responses to acidic amino acids, applied by pressure ejection, before and during hypoxia. Rat hippocampal slices were submerged in continually flowing artificial cerebrospinal fluid (ACSF) (1.5-2 ml/min) containing 95% O_2:5% CO_2. Extracellular population EPSPs were recorded in stratum radiatum of CA1 in response to stimulation of the Schaffer collateral/commissural fibers. Extracellular focal potentials (FP) were induced by pressure ejection of the NMDA-type acidic amino acid agonists N-methyl-D-aspartic acid (NMDA;100μM) and L-homocysteic acid (L-HCA;500μM) and the non-NMDA agonist willardiine (100μM). Agonists were ejected into stratum radiatum of CA1 within 100μm of the recording electrode. Hypoxia was induced by replacing the ACSF containing 95% O_2:5% CO_2 with ACSF containing 95% N_2:5% CO_2. Within 2-4 min of replacing O_2 with N_2, the population EPSP was abolished. In contrast, focal potentials induced by any of the agonists persisted for at least 30 min (the longest period of hypoxia studied). Focal potentials induced by NMDA, L-HCA, and willardiine, were reduced to 66.3 ± 6.0%, 51.6 ± 5.6%, and 64.3 ± 9.0% (Mn ± SEM) of control, respectively. The results indicate that presynaptic mechanisms, such as transmitter release, are more sensitive to hypoxia than postsynaptic receptors. Postsynaptic responsiveness remains even during prolonged hypoxia in vitro.

417.5 POSTISCHEMIC APPLICATION OF CYCLOHEXYL ADENOSINE (CHA) IN GERBILS: ENHANCEMENT OF SURVIVAL. D.K.J.E.von LUBITZ*, S.L.COHAN*, D.J.REDMOND*. (SPON.A.Raines). Dept.of Neurology, Georgetown University School of Medicine, Washington, D.C. 20007

Several authors reported that stable analogues of adenosine are potent inhibitors of glutamatergic transmission in the brain, and that A1 receptors for these compounds are particularly dense in the ischemia vulnerable regions. We have demonstrated previously that cyclohexyl adenosine(CHA), injected into the ventricles of the gerbil brain affords both qualitative and quantitative neuronal protection in the cortex, striatum and hippocampus. In the present study the impact of a single I.P. injection of CHA upon survival of severe ischemia was investigated.

57 male gerbils, 70-80g, were anesthetised with 0.5% Halothane, 33% N_2O and 72% O_2. Both carotid arteries were occluded with microaneurysm clips for 30 min resulting in forebrain ischemia. During the entire procedure blood pressure, blood O_2 concentration and cardiac rate were monitored nonivasively. The body temperature was held at 36.5-37.5°C. CHA in 10% ethanol was injected (2 mg/kg) 5 min after ischemia. The controls were injected with the vehicle.

The mortality pattern is shown in the graph below. The controls woke up 30-90 min after ischemia and suffered periods of intense seizures, dying 48 hrs later. The CHA gerbils woke up 15-24 hrs after ischemia. Initially sluggish, they regained normal behaviour within the next 24 hrs. There were no seizure episodes. 7 days after ischemia there were 63% CHA treated survivors but only 5% controls ($P < 0.001$).



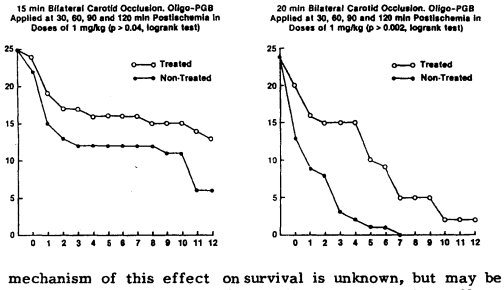
Our study shows that even a single postischemic injection of CHA considerably improves survival of an otherwise lethal ischemic episode. Further studies on the mechanism of postischemic protection by CHA are presently made in our laboratory.

This work was supported in part by the Office of Naval Research grant nr.N00014-K-0471

417.6 CEREBRAL ISCHEMIA IN GERBILS: REDUCTION IN MORTALITY FOLLOWING TREATMENT WITH OLIGO PROSTAGLANDIN B_1. S.L. Cohan, D.K.J.E. von Lubitz,* D.J. Redmond* (Spon: K.L. Dretchen) Dept. of Neurology, Georgetown University School of Medicine, Washington, D.C. 20007.

Polymeric forms of prostaglandin B_1 (PGB) may stabilize oxidative phosphorylation, act as a potent calcium ionophor (Devlin), and protect the functional integrity of mitochondria subjected to anoxic insult. Since the trimer of PGB_1 appears to have the greatest effect on mitochondria, we have studied the effect of treatment with PGB_1 trimer on survival following cerebral ischemia in the gerbils. 150 male gerbils (70-80 gm), were anesthetized with 0.5 % halothane and 33% N_20, subjected to 15 or 20 min. of bilateral cerebral ischemia by occluding both carotid arteries by a suture loop which was verified by direct inspection. Rectal temperature was maintained at 36.5-37.5°C by a heating blanket. Blood pressure, pulse, and O_2 saturation were continuously monitored during ischemia. Gerbils received either PGB_1 trimer dissolved in 4% $NaHC0_3$, 4% $NaHCO_3$ alone, or 0.9% NaCl. intraperitoneally. Animals were injected at 30, 60, 90, and 120 min. after cessation of ischemia.

Results: There were no differences in temperature, BP, pulse or O_2 saturation in the various treatment groups. The results demonstrate significant improvement in survival in animals treated with PGB_1 as compared to either control group following 15 min ($P<.04$) or 20 min ($P<.002$) cerebral ischemia:



The mechanism of this effect on survival is unknown, but may be the result of PGB_1 effects or calcium flux or protective effects on mitochondrial function. Other possible protective mechanisms include effects in free radical formation and cerebral blood flow.

Sponsored by ONR grant N00014-K-0471. Oligo-PGB_1 was supplied by Dr. G. Nelson.

417.7 THE INFLUENCE OF HYPERGLYCEMIA ON DELAYED NEURONAL CELL DEATH FOLLOWING DIFFUSE CEREBRAL ISCHEMIA IN THE RAT. K. Moszynski*, L. W. Jenkins*, W. Lewelt*, B. G. Lyeth, D. S. DeWitt*, R. L. Hayes, A. Allen*, J. Opoku*, A. Marmarou*, R. L. Keenan* and H. F. Young*. (SPON: R. Newton). Div. of Neurosurgery and Department of Anesthesiology, Medical College of Virginia/ Virginia Commonwealth University, Richmond, VA 23298-0001.

It is well documented that pre-ischemic serum hyperglycemia exacerbates ischemic brain injury. However, little data are available on ischemic neuronal cell death in hyperglycemic animals with more than 24 hours of post-ischemic recovery. The present study evaluated the effect of pre-ischemic serum hyperglycemia on delayed ischemic neuronal cell death after one to seven days post-ischemic survival in the rat.

Normally fed or fasted male Wistar rats under halothane/nitrous oxide anesthesia were subjected to 10 minutes of forebrain ischemia via bilateral carotid clamping and hemorrhagic hypotension. Two separate weight groups (250-290 g or 310-350 g) of non-fasted animals and one fasted group (270-350 g) were evaluated. EEG and evoked potentials were recorded prior to and during ischemia, 1 hour post-ischemia and 1 to 7 days post-ischemia. Morphological analysis was performed on animals perfused at 1 to 7 days of post-ischemic survival.

Fasted animals all survived 7 days and had complete qualitative recovery of electrical activity but extensive neuronal loss in the CA1 sector of the rostral third of the dorsal hippocampus. One group of fed rats (250-290 g) all survived 5 to 7 days, but demonstrated persistent deficits in electrical function. In addition to CA1 hippocampal cell loss, scattered cell death was found in other intrinsic hippocampal areas. Laminar neuronal loss in numerous cortical regions was also seen. In contrast, post-ischemic survival in fed rats over 300 g was limited to 24-48 hours at which time the animals died or were electively sacrificed due to impending death. Extensive neuronal loss was already present even after 24 hours survival in these animals and at times involved the entire hippocampus. Extensive cortical and subcortical regions demonstrated both neuronal and glial cell loss. Cell loss was also frequently asymmetrical.

These studies suggest that hyperglycemic-induced acidosis potentiates short-term and delayed neuronal loss and may be an important factor in extending the pattern of selective vulnerability to brain regions other than the hippocampus in the rat. A possible mechanism may be that acidosis impairs intraneuronal calcium buffering and enhances post-ischemic neuronal calcium toxicity secondary to abnormal agonist-receptor interactions. (Supported by NS 19550 and AHA-VA Affiliate.)

417.8 DELAYED NEURONAL NECROSIS AND POST-ISCHEMIC FLUOROCHEMICAL HEMODILUTION FOLLOWING DIFFUSE CEREBRAL ISCHEMIA IN THE RAT. W. Lewelt,* K. Moszynski,* L. W. Jenkins,* D. S. DeWitt,* A. Allen,* J. Opoku,* R. L. Keenan* and H. F. Young* (SPON: W. Rosenblum). Div. of Neurosurgery and Depart. of Anesthesiology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298-0001.

Delayed neuronal death following diffuse forebrain ischemia in rats is well documented. While neuronal connectivity and excitatory neurotransmitters have been implicated in this process, delayed changes in cerebral blood flow have not been excluded. Since serial regional cerebral blood flow (CBF) measurements over the time course of delayed neuronal death would be prohibitively labor intensive, an alternative approach is to provide therapeutic manipulations to enhance post-ischemic CBF and minimize blood flow perturbations. The present study used normovolemic hemodilution with the low viscosity oxygen carrier perfluorotributylamine (FC-43) in an attempt to improve brain bioelectrical function and structural integrity following transient incomplete cerebral ischemia.

Fasted Wistar rats, anesthetized with halothane and nitrous oxide, were subjected to 10 minutes of cerebral ischemia by bilateral carotid artery clamping and simultaneous hemorrhagic hypotension. One group was reinfused with normal blood to restore circulation and the other was reinfused with FC-43 (50% reduction in hematocrit). EEG, somatosensory and auditory evoked potentials were recorded prior to and during ischemia, 1 hour post-, and 7 days post-ischemia. All animals were perfused for morphological analyses after 7 days survival.

The EEG showed 50% recovery in non-treated and 75% recovery in FC-43 receiving animals during the first hour of recirculation. EEG appeared qualitatively normal by day 7 in both groups. There was also a trend for a quicker recovery of evoked potentials in FC-43-treated animals but both groups had qualitatively recovered evoked potentials by day 7. There was extensive pyramidal cell loss of the hippocampal CA1 sector in the ventral third of the dorsal hippocampus of both groups with no overt differences seen between groups.

Hemodilution appeared to improve the acute recovery time of electrical activity but it had no effect on the process of delayed neuronal death in fasted rats. From these data it would appear that immediate post-ischemic hemodilution has no beneficial effect on delayed neuronal death in fasted rats which suffer incomplete cerebral ischemia. (Supported by AHA-VA Affiliate and NS19550.)

417.9 KAINATE (KA) DOSE-RESPONSE CURVE: CHANGES IN RAT MOTOR AND NEUROPATHOLOGIC PATTERN. R.E. Ruth and A.A. Seimen*. Inst. Study Dev. Disabil., and Committee on Neurosci., Univ. Il., Chicago 60608.

Systemic KA injections induce limbic motor seizures and ultimately, widespread limbic brain damage. Using convulsive doses of KA slightly below the LD-50 (1X-dose), we previously observed that few neurons are irreversibly injured prior to the onset of motorically defined status epilepticus (SE). However within 5 minutes of SE onset neuron death commences in several amygdaloid and closely apposed nuclei. As SE continues the severity of damage increases in these regions, while little (if any) irreversible damage is apparent within the hippocampal formation after 30 minutes of SE.

We have now examined additional cases that sustained 10--20 minutes of motorically defined SE, induced by injections of kainate at approximately 2X and 3X the LD-50. The motor pattern during the first 20 minutes of SE qualitatively changed with increasing KA dosage. At the 1X-dose SE onset was preceded by staring, wet-dog shakes, minor head automatisms and brief episodes of rearing/forelimb clonus; SE consisted of repetitive, pronounced head automatisms punctuated by frequent and severe rearing episodes. At the 2X-dose wet-dog shakes were completely suppressed, the frequency of rearings declined, and a new behavior emerged during SE: intense escape behavior. At the 3X-dose escape behavior no longer appeared, being replaced by prominent tonic and opisthotonic components.

Preliminary neuropathologic analysis of brain damage in these cases yielded somewhat unexpected results. As shown previously, within 30 minutes of SE onset no apparent damage was present in the hippocampal formation from the 1X-dose. At the 2X-dose there was near-total loss of CA3 pyramidal cells occupying the middle 1/3 of the septotemporal axis, with patchy CA3 damage in the septal 1/3 of the axis. At the 3X-dose the damage expanded to include the entire septal 2/3 of field CA3 and corresponding parts of the hilus (CA4). Curiously the temporal 1/3 of field CA3 was spared at all dosages, and the remaining hippocampal fields also appeared to be intact.

In contrast, destruction within and surrounding the amygdaloid region was moderate to severe at the 1X-dose, light to moderate at the 2X-dose, and nonexistent to light at the 3X-dose. The results suggest that (i) varying the KA dosage produces qualitatively different motor and neurologic sequelae; (ii) the temporal 1/3 of the hippocampal formation is highly resistant to the actions of KA; and (iii) KA-induced damage to the hippocampus may suppress subsequent amygdaloid damage and vice-versa.

417.10 NEUROGENIC MECHANISMS IN EXPERIMENTAL VASOSPASM. R. Cach*, T. Smock, D. Albeck* and E. Florence*. Behavioral Neuroscience Program, Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Penetrating microvessels such as those found in the rat hippocampal slice preparation provide the bulk of cerebrovascular resistance and hence changes in their tone could have substantial impact on cerebral blood flow. Penetrating intraparenchymal vessels in the slice have been shown to constrict following the application of coagulated blood serum or in response to a blood-borne eicosanoid, thromboxane b_2 (TXB_2) at concentrations achieved in experimental ischemia (1.35 nM, Cach et al., Brain Res., in press). Given the possibility of a substantial neurogenic component in experimental vasospasm we have examined the electrophysiology of blood serum and TXB_2 responses in an attempt to clarify the relationship between neural activity and microvascular constriction.

Rat hippocampal brain slices were used for time-lapse microphotography of individual vessels (avg. lumenal diameter 17 microns). Slices were exposed to normal Ringer solution or normal Ringer solution containing 1 µM tetrodotoxin (TTX) using a blind paradigm. Thirty minutes of incubation in TTX was found to be ample time for complete blockade of all neural activity as determined for field potentials taken from the slices. Vessels in slices exposed to thirty minutes of TTX application did not respond to subsequent serum application (n=7), but in slices not exposed to TTX vessels responded invariably to serum application (n=8).

To further examine the electrophysiology of the neural response to plasma extracellular field potentials were recorded from the granule layer of the dentate gyrus following perforant path stimulation (7-13 v, 60-90 µsec pulse duration). Fifteen to twenty minutes of plasma application to the slice either had no effect or decreased the population spike and epsp amplitude (n=8). The same amount of exposure to 1.35 nM TXB_2, however, was found to increase both the population spike by 26% (± 8) and the compound epsp amplitude by 22% (± 8, n=9).

Sodium-mediated neural action potentials are blocked by TTX, but smooth muscle action potentials are not sensitive to this toxin. The ability of TTX to block the vascular constriction normally produced by plasma suggests the probable involvement of neural mechanisms in the plasma induced vascular effects. The results of the field potential records support a role for the blood plasma spasmogen TXB_2 in increasing neural excitability, although the electrophysiology results with whole plasma suggest that some subpopulation of vasoregulatory neurons more sensitive to TXB_2 than granule cells may mediate the spasmogenic action. In attempting to fully describe the events leading to cerebral vasospasm and resulting cerebral ischemia, the results described here must be more fully elucidated and examined in conjunction with the action of humoral factors.

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417.11 THE DEVELOPMENT AND DISTRIBUTION OF BRAIN DAMAGE AFTER CARDIAC ARREST IN THE RAT. P.H. Schwartz, R.D. Gottesman*, B.E. Dwyer*, and C.G. Wasterlain*. Div. Pediatric Intensive Care, Childrens Hospital of Los Angeles; Epilepsy Research Laboratory, Veterans Administration Medical Center, Sepulveda, CA 91343; and Department of Neuroscience, Brain Research Institute, UCLA.

Using the model of cardiac arrest that we have described (Soc. Neurosci. Abstr. 12:63, 1986), we investigated the time-course of the development and the distribution of brain damage after ten minutes of arrest. Rats (250-400g) were anesthetized with ketamine and ventilated. Femoral vessels were cannulated for measurement of blood pressure and gases and administration of drugs. After neuromuscular blockade with succinylcholine a transcutaneous intracardiac dose of cold KCl was injected to produce instant fibrillation resulting in the complete loss of blood pressure. After ten minutes the animals were resuscitated. Animals were sacrificed 0 and 30 minutes, 4 and 24 hours, and 3 and 6 days later by pentobarbital anesthesia and aortic perfusion with neutral buffered formalin. The brains and spinal cords were embedded in paraffin. Semi-serial 8u sections stained with H+E were examined under the light microscope. Six days after arrest damage was confined mostly to the CA1 and subiculum of the hippocampus, the septohippocampal nucleus, layers 3-5 of lateral neocortex, and the Purkinje cells of the cerebellum. Occasional microhemorrhages or foci of extensive neuronal necrosis were seen in the hippocampus, the cerebellar nuclei, and the spinal cord. No damage was seen in the striatum, the lateral septum, or the thalamus. The early stages of damage (dark cells) were seen at four hours in the cerebellum and, to a lesser extent, the cortex. Since dark cells were seen in all layers of the cortex, dark cells do not necessarily represent an irreversible stage of damage. At 24 hours necrotic (eosinophilic) neurons were apparent in layers 3-5 of the cortex and the Purkinje cell layer of the cerebellum. Even up to 24 hours after the insult only an occasional necrotic pyramidal cell in CA1 and the septohippocampal nucleus and a few necrotic interneurons in CA3 and the hilus were seen. The full extent of damage to the septohippocampal nucleus, CA1, and the subiculum did not become apparent until three days after the insult. These results support the concept of delayed neuronal necrosis in the CA1 and subiculum as well as the septohippocampal nucleus and also demonstrate the selective vulnerability and rapid development of necrosis of the cerebellar Purkinje cells after cardiac arrest.

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417.12 EFFECT OF FOCAL CEREBRAL EDEMA ON PARAVASCULAR FLUID/TRACER MOVEMENTS IN THE BRAIN. O.R. Blaumanis*, M.L. Rennels and P.A. Grady. Depts. of Neurology and Anatomy, Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

The intraparenchymal vascular network supplying the brain is surrounded by paravascular fluid pathways that communicate with the subarachnoid space (SAS). These pathways include the perivascular spaces (PVS) around arterioles, the basal laminae (BL) around capillaries, and the PVS around veins. They can be demonstrated by infusion of the enzyme, horseradish peroxidase (HRP), into the SAS; within minutes, the tracer enters the PVS around arterioles and spreads through the BL of capillaries to reach the PVS of veins. This unidirectional tracer movement appears to reflect a convective fluid flow occurring in parallel to the vasculature, that is facilitated by pulsations of penetrating arterioles (Brain Res., 326:47). Tracer distribution through this system would presumably be affected by experimentally induced abnormalities in the intracerebral fluid environment. This study was undertaken to test whether paravascular tracer distribution is blocked in areas of focal vasogenic edema. A parietal lobe lesion was created unilaterally by application of a cold probe (liquid nitrogen temp.) to the exposed skull of anesthetized cats. The animals were reanesthetized after 24 hrs. and injected IV with 10 cc of 2% Evans blue (EB) in Hanks' solution (allowed to circulate 30 min.) A needle was then inserted into the cisterna magna and 1.0 ml CSF was drained and replaced with an equal volume of 4% HRP (Sigma, Type II) in Hanks' solution. After 10 min., animals were perfused with aldehyde solutions. Extravasation of EB revealed a 1.0 cm stained region on the lesioned hemisphere extending through the cortex and an area of blood-brain barrier disruption and edema in the underlying white matter. Brain sections were then incubated for HRP localization using diaminobenzidine or tetramethylbenzidine. In the lesioned hemisphere, tracer was excluded from a broad area beneath the lesion; reaction product was limited to surrounding areas of the vasculature and white matter. By contrast, the vascular network and white matter of the intact hemisphere were densely labelled. These findings support our hypothesis of an ongoing paravascular fluid circulation in the CNS and may have significance for studies of the development and/or clearance of cerebral edema.

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417.13 AGE, GENDER AND BODY POSITION AFFECT CAROTID ARTERY BLOOD FLOW VELOCITY IN RESPONSE TO VALSALVA STRAIN IN HUMANS. B. Cimprich*, B.L. Metzger*, and B.A. Therrien, The University of Michigan, Ann Arbor, MI 48109

Marked shifts in cerebrovascular blood flow velocity and pressure in response to straining (Valsalva Maneuver) are life-threatening in individuals with cerebrovascular disease (CVD). It is known that the incidence of CVD increases with age, and there is some evidence to suggest that gender influences the specific form of CVD pathology. In contrast, little is known about reducing or preventing marked hemodynamic shifts. In this study we examined the effects of age, gender and one potential modifier (body positioning) on carotid artery blood flow velocity (CABFV) during straining.

Subjects strained by blowing into a pressure gauge meter to 40 mmHg for 10 seconds. CABFV was measured by noninvasive Doppler technique continuously over phases of the Valsalva Maneuver (baseline, strain, overshoot). 80 younger males and 65 younger females (30-55 yrs.) were tested in 5 positions (flat, side, 30^{o} and 70^{o} upright, and chair) and 19 older males and 47 older females (over 55 yrs.) were tested in the flat and 70^{o} upright positions. CABFV changed significantly during strain ($F = 35.41$; $p<.001$) and following release (overshoot) of strain ($F = 13.17$; $p<.001$) as a function of body position. During the strain, significantly greater reduction in CABFV was seen in the more reclining as compared to upright positions ($\bar{X}$ = -65% vs -52% respectively; $p<.05$). At overshoot, greater increases in CABFV were also observed in the flatter as compared to more upright positions ($\bar{X}$ = 313% vs 213% respectively; $p<.05$).

Both age and gender significantly affected CABFV in response to straining. Changes in CABFV were significantly more intense in older as compared to younger subjects. Specifically, older males had greater reductions in CABFV during the period of strain while lying flat than did older females ($\bar{X}$ = -76% vs -68% respectively; $p<.05$) and all younger subjects ($\bar{X}$ = -76% vs -62%; $p<.05$) as well as greater increases during overshoot than did younger subjects ($\bar{X}$ = 397% vs 282%; $p<.05$). A gender difference was also identified in younger subjects. Young females, when placed upright in a chair, had less reduction in CABFV during strain as compared to males ($\bar{X}$ = -42% vs -49% respectively; $p<.05$) and less increase at overshoot ($\bar{X}$ = 120% vs 188%; $p<.05$).

We conclude that CABFV responses to straining become significantly more intense with age and that gender differences exist. Furthermore, we suggest that the intensity of CABFV changes is modified by body position and can be lessened by more upright positions.

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417.14 INCREASED CALCIUM-ACTIVATED NEUTRAL PROTEINASE ACTIVITY IN CEREBRAL CORTEX FOLLOWING ISCHEMIA. T. H. Liu*, A. K. Chakrabarti*, N. L. Banik, E. L. Hogan, C. Y. Hsu. Department of Neurology, Medical University of South Carolina, Charleston, SC 29425.

Calcium may play a pivotal role in post-ischemic tissue injury. One of the calcium-mediated biochemical processes which may lead to tissue damage is through calcium-activated neutral proteinase (CANP). In a focal ischemic stroke model in the rat (S. T. Chen et al, Stroke 17:738, 1986,) we have noted that the calcium accumulation in cerebral cortex is correlated with the infarct size (S. T. Chen et al, Neurology, in press). In the present studies we determined cortical CANP activity after ischemia. Focal cerebral ischemia in cortex of the right middle cerebral artery (MCA) territory was induced by ligation of the right MCA and temporary clamping of both common carotid arteries (CCAs) for 30, 60 or 90 minutes. In sham-operation controls, the arteries were isolated only. CANP activity was assayed using ^{14}C-azocasein (N. L. Banik et al, Brain Res., in press). CANP activity was $12.1 \pm 0.6 \times 10^{3}$ cpm/mg protein (mean $\pm$ SEM, N = 4) in sham-operated rats and did not change between day 1 and 14 post surgery. In rats with 90 min clamping of both CCAs CANP activity was 30.2 ± 2.4; 45.3 ± 2.5, 134.6 ± 15.3; and 103.5 ± 5.1; 1, 2, 7 and 14 days respectively after ischemia. Increase in CANP activity was less when duration of CCA clamping was reduced (30 min: 16.4 ± 0.98; 60 min: 48.4 ± 1.6, 7 days post ischemia). The potential roles of calcium accumulation and increase in CANP activity in ischemic injury are suggested by the observation that post-ischemic degradation of neurofilament protein in this stroke model evolved over a period of 2 weeks (%loss of 150 K neurofilament protein: day 1, 7.7 ± 2.5; day 3, 19.1 ± 3.0; day 7, 38.3 ± 6.3; day 14, 47.5 ± 4.7) correlating closely with increase in calcium content and CANP activity. Results from these studies are consistent with the hypothesis that calcium activation of CANP may be one of the biochemical events leading to post-ischemic degradation of CNS cytoskeleton proteins.

417.15 INHIBITION OF FREE POLYUNSATURATED FATTY ACID ACCUMULATION IN MOUSE BRAIN DURING ELECTROCONVULSIVE SHOCK AND ISCHEMIA BY THE SPECIFIC PAF ANTAGONIST, BN52021. Dale L. Birkle*, Pierre Braquet*[1], Nicolas G. Bazan. Louisiana State University Eye Center, New Orleans, LA 70112. [1]Institute Henri Beaufour, Paris, France

We have investigated the effects of the specific PAF (1-alkyl-2-acetyl-glycerophosphocholine) antagonist, BN52021, on the accumulation of free fatty acids (FFA) and diacylglycerols (DG), and the loss of fatty acids from polyphosphoinositides (polyPIs) in the mouse brain. Two models known to cause rapid breakdown of polyPIs and accumulation of FFA and DG were studied; electroconvulsive shock (ECS) (50 V, 200 msec train, 0.5 msec pulse, 100 pps), and post-decapitation ischemia. Mice (BALB/C) were pretreated with 10 mg/kg BN52021 (i.p.) 30 min prior to ECS or decapitation. ECS-treated mice were killed by high power microwave irradiation (6.5 kW for 0.6 sec) 30 sec after the ECS stimulus, during the clonic phase of the seizure. Post-decapitation ischemia was for exactly 1 min. Lipid extracts were prepared from cerebrum, fractionated by TLC, the fatty acid methyl esters (FAMEs) prepared by methanolysis, and quantitated by capillary GLC. In vehicle-treated mice (DMSO, 275 mg/kg), ECS caused a marked accumulation of FFA and DG, and a loss of mainly stearic (18:0) and arachidonic (20:4) acids from polyPIs. Pretreatment with the PAF antagonist, BN52021, decreased the accumulation of palmitic acid (16:0), 18:0, 20:4 and docosahexaenoic acid (22:6) in the FFA pool, and the loss of 20:4 and 22:6 from polyPIs, with no effect on the content or composition of fatty acids in DG. BN52021 had no effect on basal levels of FFA, but slightly increased the basal level of 18:0 and 20:4 in DG. One minute post-decapitation ischemia induced a massive accumulation of FFA and DG in the mouse brain. Pretreatment with BN52021 attenuated the accumulation of free 20:4 and 22:6, and decreased the content of oleic acid (18:1) and 20:4 in DG. This data indicate that PAF may be involved in the injury-induced activation of phospholipase A_2, which mediates the release of polyunsaturated fatty acids in brain. Alternatively or additionally, as deacylation of 20:4 and 22:6 is part of the PAF cycle, BN52021 may have an effect on the activation or inactivation of PAF in brain, thereby indirectly influencing the level of free fatty acids. (Supported in part by NS23002 from NINCDS).

417.16 **OXYGEN FREE RADICALS AND XANTHINE OXIDASE IN CEREBRAL ISCHEMIC INJURY IN THE RAT.** J.S. Beckman[1]*, T.H. Liu[2]*, E.L. Hogan[2]*, B.A. Freeman[1]* and C.Y. Hsu[2]. Depts. of Anesthesiology and Biochemistry, University of Alabama at Birmingham, Birmingham AL 35233 and Dept. of Neurology, Medical College of South Carolina, Charleston, SC 29425-2232.

The protective effects of the oxygen free radical scavengers, polyethylene glycol-conjugated superoxide dismutase (PEG-SOD; a superoxide scavenger) and PEG-catalase (a H_2O_2 scavenger), were investigated in a rat stroke model. Ligation of the right middle cerebral artery (MCA) with 10-0 silk at the level of the rhinal fissure and temporary clamping of both carotid arteries for 90 min yields a focal infarct of 188 ± 28 mm^3 (mean ± SEM) restricted to the right cortex. Ninety min of carotid clamping gives a sub-maximal but highly reproducible infarction volume. In a randomized, blinded study, the volume of cortical infarction was reduced by 30% (to 132 ± 15 mm^3) in rats pretreated IV with 10,000 units/kg each of PEG-SOD plus PEG-catalase, whereas infarct volume in rats pretreated with inactivated PEG-SOD plus PEG-catalase was 187 ± 13 mm^3 ($p<0.01$; $n=18$). The area of infarction in the inactive enzyme group was the same as historical controls, indicating that the protection obtained with active PEG-SOD plus PEG-catalase resulted from scavenging of superoxide and/or H_2O_2 rather than a nonspecific protein artifact. Injection of either active or inactive PEG-SOD plus PEG-catalase had no effect on heart rate, systemic arterial blood pressure, or blood gasses. One tissue source of superoxide and H_2O_2 may be the ischemia-induced conversion of xanthine dehydrogenase to the free radical-producing form, xanthine oxidase. Three hours after release of the carotid clamps, the fraction of xanthine dehydrogenase converted to the free radical-producing oxidase increased by 17% in the MCA territory of the right cortex but not in the left cortex. Total xanthine dehydrogenase plus oxidase activity remained constant, being 6 to 7 nmol product formed min^{-1} g^{-1} cortex in control, ischemic and reperfused rat brain. Between 40 to 80% of brain xanthine dehydrogenase plus oxidase is localized within the microvasculature, based upon the relative enrichment of xanthine dehydrogenase compared to alkaline phosphatase in isolated microvessels. Because endothelium constitutes only 0.5% of brain volume, the amount of xanthine oxidase formed in ischemic right cortex can generate 70 and 170 μM/min of superoxide and H_2O_2 respectively within the endothelium. Equivalent concentrations of xanthine plus xanthine oxidase applied to cultured endothelial cells will rapidly destroy barrier function and cause cell lysis. Thus, we hypothesize that free radical formation by xanthine oxidase contributes to the breakdown of the blood-brain barrier that occurs several hours after reperfusion of ischemic brain tissue, and this could account for the protection obtained by PEG-SOD and PEG-catalase injected into the circulation.

417.17 ENTORHINAL CORTICAL LESION PARTIALLY PREVENTS HIPPOCAMPAL CELL DEATH AFTER TRANSIENT FOREBRAIN ISCHEMIA IN THE GERBIL. T.M. Lasner*, B.J. Crain and J.V. Nadler. Depts. Anatomy, Pathology and Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Transient forebrain ischemia in the Mongolian gerbil produces a spatially and temporally selective pattern of neuronal degeneration in the hippocampal formation. Ischemic cell death evolves within 6 h in area CA4 and within 24-48 h in areas CA2 and CA1a. In area CA1b, however, neuronal degeneration is delayed for 2-4 d. The mechanism for delayed neuronal death in area CA1b is unknown, but it has been suggested that the activation of excitatory hippocampal afferent pathways may be crucial. We have tested this hypothesis by examining the effect of a unilateral entorhinal cortical (ERC) lesion on the neuropathology produced by a brief bilateral carotid occlusion.

Adult male gerbils received a unilateral electrolytic ERC lesion. Control animals received a sham lesion (sham-ERC), for which the electrode was lowered into the brain but no current was passed. Seven days later, both experimental and control animals were anesthetized with 2.5% halothane and the carotid arteries were occluded bilaterally for 5 min. Four days after the carotid occlusion, the brains were removed and sections were stained by silver impregnation and with cresyl violet. To quantitate effects of the ERC lesion, neurons were counted in three cresyl violet-stained sections cut through the dorsal hippocampal formation of each experimental, control and untreated animal.

In animals subjected to a sham-ERC lesion, only 1% of the CA1b neurons on either side of the brain survived the ischemic insult, and only 3% of these neurons contralateral to the ERC lesion survived. In contrast, 31% of CA1b neurons survived on the side of the brain ipsilateral to the ERC lesion. Cell counts revealed no significant effects of the ERC lesion on neuronal degeneration in area CA4 and no protective effects were evident in areas CA2 and CA1a.

Thus an ERC lesion selectively protected neurons of area CA1b from ischemic cell death. The degree of protection afforded to CA1b neurons was, however, much less in the gerbil than has been reported in the rat. If synaptic excitation is crucial for ischemic cell death in general, then the exclusive protection afforded by an ERC lesion to CA1b neurons points to involvement in the damage of the medial temporo-ammonic tract, rather than the perforant path. Alternatively, synaptic excitation may only be crucial for the most delayed form of ischemic cell death. (Supported by NIH grant NS 06233.)

417.18 ELECTRON MICROSCOPIC STUDY OF THE GERBIL DENTATE GYRUS AFTER TRANSIENT FOREBRAIN ISCHEMIA. B.J. Crain, D.A. Evenson*, K. Polsky*, D.R. Armstrong and J.V. Nadler. Depts. Anatomy, Pathology and Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Our previous silver impregnation study demonstrated a discrete pattern of neuronal degeneration after brief forebrain ischemia in the Mongolian gerbil (Soc. Neurosci. Abstr., 12, 265). A novel finding was that terminal-like silver granules could be demonstrated in the outer two-thirds of the dentate molecular layer (the perforant path terminal zone) 1-3 d after a bilateral carotid occlusion, even though neither the cell bodies of origin of the perforant path nor the dentate granule cells were destroyed. In an effort to discover the identity of these degenerating structures, we performed electron microscopic studies of the dentate gyrus.

Gerbils were anesthetized with 2.5% halothane and the carotid arteries were occluded bilaterally for 5 min. The animals were sacrificed either 24 or 48-51 h later and the hippocampi were processed for electron microscopy. Photomontages were constructed through the entire width of the ventral leaf of the rostral dentate gyrus, from the polymorph layer to the pial surface.

At 24 h after the occlusion, some enlarged and electron lucent synaptic boutons were observed in the perforant path terminal zone. However, electron dense structures were rare or absent, except in the zone immediately above the granule cell layer. These degenerating structures appeared to be postsynaptic, probably the dendritic spines and shafts of hilar neurons. Electron dense dendritic profiles were frequently observed in the polymorph layer, but presynaptic structures in this layer appeared normal. There was some evidence of phagocytosis by both astrocytes and microglia. We also observed some increase in astrocyte space in both polymorph and molecular layers.

At 48-51 h after the occlusion, numerous small, scattered electron dense profiles appeared in the perforant path terminal zone. Some of these had already been engulfed by astrocytes. Although a few of these profiles could be identified as degenerating dendritic spines, the majority were not clearly identifiable. In the polymorph layer, astrocyte space, electron dense dendritic profiles and evidence of phagocytosis had all increased compared with 24 h survival. In addition, many mossy fiber boutons appeared watery and enlarged. Most of these boutons were found to be contacting electron dense dendritic spines. The dentate granule cells appeared undamaged.

Watery, enlarged mossy fiber boutons have been associated with greatly increased synaptic activity. However, preliminary electrographic studies revealed no evidence of hippocampal seizure activity within 48 h after the occlusion. The abnormal appearance of these boutons may be related to the degeneration of postsynaptic cells. (Supported by NIH grant NS 06233.)

417.19 THE EFFECTS OF ISCHEMIA ON PROTEIN PHOSPHORYLATION IN RABBIT SPINAL CORD. A. Kochhar*, J. A. Zivin, and T. Saitoh, Dept. of Neurosciences, School of Medicine, Univ. of California, La Jolla, CA 92093.

Protein phosphorylation systems regulate many diverse intracellular functions in nervous tissue. The physiological activity of substrate proteins depends on their state of phosphorylation, which is determined by the relative activities of protein kinases (which phosphorylate substrate proteins) and phosphoprotein phosphatases (which dephosphorylate phosphoprotein substrates). We examined the effects of ischemia on protein phosphorylation in rabbit spinal cord. Spinal cords were rapidly excised and frozen from control animals or after 1 hr of ischemia which produces irreversible neurologic damage. Tissue samples were homogenized and centrifuged to separate the membrane rich and cytosolic fractions. In endogenous protein phosphorylation assays, membrane and cytosolic fractions were incubated under phosphorylating conditions, in the presence or absence of appropriate protein kinase activators. Proteins were separated by SDS-polyacrylamide gel electrophoresis, stained with Coomassie Blue, and phosphorylated proteins were detected by autoradiography. One hr of ischemia did not affect endogenous phosphorylation mediated by the cyclic AMP-dependent protein kinase system. However, the phosphorylation pattern of both calcium-dependent systems, Ca^{2+}-calmodulin-dependent protein kinase and Ca^{2+}-phospholipid-dependent protein kinase was altered in the ischemic animals. A 31 kDa phosphoprotein substrate was absent in ischemic cord and phosphorylation of three other substrates (21 kDa, 45 kDa, and 87 kDa) was markedly reduced. These studies show that ischemia selectively inhibits calcium-dependent protein kinase systems. Alterations of these crucial regulatory systems may be responsible, at least in part, for irreversible cell death in ischemia.

417.20 ROLE OF ARACHIDONIC ACID IN THE MITOCHONDRIAL RESPONSE TO CEREBRAL ISCHEMIA IN THE RAT. L. Hillered* and P.H. Chan, Brain edema Clin. Res. Center, Univ. of California, Sch. of Med., San Francisco, CA 94143.

Arachidonic acid (20:4), liberated from membrane-bound phospholipids, has been implicated in the pathophysiology of ischemic cell damage in the brain. In search for a biochemical correlate to the well known ability of 20:4 to induce brain edema, we have recently reported on profound adverse effects of free 20:4 (in concentrations relevant to cerebral ischemia) on brain mitochondrial function in vitro (Hillered, L., Chan, P.H., J. Neurosci. Res., In Press).

The present study was undertaken with the objective of testing the hypothesis that free 20:4 is responsible for the well known functional impairment of rat brain mitochondria isolated following ischemia. Mitochondria were isolated following 15 and 30 min of postdecapitative ischemia in the absence of bovine serum albumin (BSA) and their respiratory function measured polarographically with or without the addition of BSA to the reaction medium. Ischemia was, as expected, associated with a marked inhibition of phosphorylating and uncoupled respiration. There was also a small increase in resting respiration (uncoupling effect). Addition of BSA to the mitochondrial preparations resulted in a significant (approx. 20%) improvement of phosphorylating and uncoupled respiration and a reversal of the uncoupling effect. Measurement of the free fatty acid content of the mitochondrial preparations revealed a 6-8 fold increase in 20:4 during ischemia and that 20:4 was removed by treatment with BSA. Palmitic (16:0) and stearic (18:0) acid were also increased during ischemia (30 and 40%, respectively) but not removed by the BSA treatment.

Although our recent in vitro data (Hillered, L., Chan, P.H., J. Neurosci. Res., In Press) suggest that free 20:4 may be deleterious to mitochondrial ATP production in vivo during (incomplete) ischemia, it is concluded from the present results that the dysfunction of brain mitochondria isolated following ischemia may only to some extent be attributed to free 20:4.

DISEASES OF THE NERVOUS SYSTEM III

418.1 ENHANCEMENT OF NEUROLOGICAL RECOVERY AND SURVIVAL IN EXPERIMENTAL HEAD INJURY BY THE 21-AMINOSTEROID U74006F, A NOVEL AND POTENT INHIBITOR OF IRON-DEPENDENT LIPID PEROXIDATION. P.A.Yonkers*, J.M.McCall*, J.M.Braughler and E.D.Hall, CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001

The ability of a novel and extremely potent inhibitor of iron-dependent CNS tissue lipid peroxidation, U74006F (21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16α-methylpregna-1,4,9(11)-triene-3,20-dione, monomethane sulfonate) to enhance the early neurological recovery and survival of male CF-1 mice after a severe concussive head injury was investigated. Unanesthetized male CF-1 mice were subjected to a 900 g-cm head-injury produced by a 50 g weight which was dropped 18 cm. This concussive injury resulted in immediate unconsciousness (loss of righting reflex) in all animals and death in approximately 30%. Survivors received a tail vein injection of either vehicle or U74006F (0.001-30 mg/kg) within 5 minutes post-injury. Their neurological status was evaluated 1 hour later using a grip test. Administration of a single i.v. dose of U74006F resulted in a significant improvement in the 1 hour post-injury neurological status (grip test score) over a broad dose range (0.003-30 mg/kg) and by as much as 168.6%. A 1 mg/kg i.v. dose given within 5 minutes and again at 1.5 hours after a severe injury, in addition to improving early recovery, also increased the 1 week survival to 78.6% compared to 27.3% in vehicle-treated mice ($p<0.02$). The compound was also effective in enhancing early recovery after a more moderate injury. This study demonstrates that early treatment after severe concussive head injury with a potent inhibitor of iron-dependent lipid peroxidation can significantly benefit the injured brain such that both early neurological recovery and long-term survival are promoted.

418.2 **THE 21-AMINOSTEROID, U-74006F, ENHANCES NEUROLOGICAL RECOVERY IN A MODEL OF SPINAL CORD COMPRESSION INJURY.** D.K. Anderson(a), J.M. Braughler(b), E.D. Hall(b), T.R. Waters*(a), J.M. McCall*(b), and E.D. Means(a). (a)Cincinnati VA Medical Center, Cincinnati, OH 45220; (b)CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

This study was designed to assess the dose-response characteristics and effectiveness of U-74006F to enhance functional recovery in cats subjected to compression trauma of the upper lumbar (L2) spinal cord. This compound is one of a series of novel 21-aminosteroids that lack glucocorticoid receptor mediated activity and are potent inhibitors of lipid peroxidation. At 30 min following injury, treatment was initiated with either vehicle (sterile water) or one of 8 i.v. doses of U-74006F. Initial doses of U-74006F ranged from 0.01 mg/kg to 30 mg/kg. Subsequent dosing consisted of additional i.v. bolus injections followed by a continuous 42h i.v. infusion. Over the 48h treatment period cats received a total U-74006F dose of 0.048 mg/kg to 160 mg/kg. Treatments were randomized and investigator blinded. Animals were evaluated weekly for neurologic recovery based upon an 11 point behavioral scale. At 4 wk after injury, the spinal cords were removed for histological assessment. With the exception of one dose (16 mg/kg/48h), total doses of 1.6 to 160.0 mg/kg resulted in nearly 75% return of normal neurological function by 4 wk after injury. Lower doses of 0.16 and 0.48 mg/kg/48h were associated with approximately 50% return of normal function which was not significantly better than vehicle-treated controls. The lowest dose tested (0.048 mg/kg/48h) was indistinguishable from vehicle-treated cats that recovered only 20% of their preinjury neurological function by 4 wk. Transverse sections of the lumbar spinal cord were stained with luxol fast blue for myelin and evaluated for cavity dimensions. The section of cord with the largest cavity was digitized using a Videoplan (Zeiss) Image Analysis device and programmed calculation. The outside and junction between myelin and glial-cavity of the cord were digitized and % of cord (cavity/cord x 100) occupied by cavity and glia was calculated. Mean cavity dimension was 49% for controls and 27% for the treatment groups. These results demonstrate that over a 100-fold range of doses, U-74006F is remarkably effective in promoting function recovery in cats subjected to compression trauma of the spinal cord.

418.3 **DEVELOPMENT OF AXONAL PATHOLOGIES AFTER GRADED SPINAL CORD CONTUSIVE INJURY IN THE RAT.** J. R. Wrathall, L. Verma* and B. M. Kolodner*. Department of Anatomy and Cell Biology, Georgetown University, Washington, D.C. 20007.

We have previously reported quantitative light microscopic studies on tissue loss after graded spinal cord contusive injury (*Soc. Neurosci. Abstr.* 11:1168, 1985). In order to examine early pathological changes more closely, particularly the development of axonal pathologies, we have now analyzed plastic-embedded tissue at the light and electron microscopic level and used stereological techniques to initiate a quantitative description of axonal loss with time.

Adult Sprague-Dawley rats were anesthetized and subjected to standardized contusive injuries that produce mild or severe chronic functional deficits (*Exp. Neurol.* 88:108, 1985). At 15 min, 2, 4, 24 and 48 hr after injury, tissue was fixed by perfusion with 2% glutaraldehyde, 2 % paraformaldehyde and 2mM $CaCl_2$ in 0.1 M cacodylate buffer (pH 7.3) and 200 μm cross-sections of cord at the injury site prepared with a tissue chopper. Sections representing the epicenter (region of maximal damage) were osmicated with 1% osmium-1.5% potassium ferricyanide, stained en bloc with uranyl acetate, dehydrated and flat embedded in araldite. Semithin 1 μm sections of the entire cross section were analyzed and the blocks trimmed to prepare thin sections of ventral, lateral and dorsal segments of the cord section. Thin sections were examined in a JEOL 100S electron microscope. For quantitative analysis, micrographs were prepared to sample the white matter near the ventral median fissure, axons counted, and the micrographs subjected to stereological analysis using a test grid to assign numerical designators to all elements at the grid intersections. Data were accumulated and the percentage of occurrence of each element calculated and compared at the different times after contusive injury.

The results indicate severe gray matter pathology, including neuronal chromatolysis, pyknosis and loss by 15 min after both mild and severe injury. Hemorrhage was more extensive in gray than white matter and more extensive after mild versus severe injury. Edema, as indicated by overall swelling and increased interstitial space, was greater after severe than mild injury. Severe axonal pathologies appeared more rapidly and were more extensive after severe than mild injury. After both degrees of injury there was a central to peripheral radial gradation of axonal pathology with greater numbers of more normal axons at the periphery of the cord. However, pathological axons were seen even at the extreme edge of the cord immediately adjacent to normal-appearing small myelinated axons. By comparison with normal white matter, larger myelinated axons appeared to be lost preferentially. (Supported by NIH-NINCDS, NO1-NS-2-2310).

418.4 **LACK OF EFFECT OF NALOXONE OR TRH ON FUNCTIONAL DEFICIT IN RATS WITH CONTUSIVE SPINAL CORD INJURIES.** D.W. Hoovler, E. Fujii*, E. Scheckowitz* and J.R. Wrathall. Department of Anatomy and Cell Biology, Georgetown University, Washington, D.C. 20007.

Presently, there is an ongoing search for a drug or treatment therapy which will aid in the alleviation of the motor and sensory deficits seen after spinal cord injury. Naloxone and thyrotropin releasing hormone (TRH) have shown promise in several spinal cord injury studies in which cats were used as experimental subjects (*J. Neurosurg.* 55:209, 1981; *Neurology* 33:673, 1983). The present study was designed to examine the effects of naloxone and TRH administration on the functional deficit, as assessed behaviorally, in adult rats following a standardized, reproducible, moderate spinal cord contusive injury.

Adult Sprague-Dawley rats (200-225 grams) of both sexes were anesthetized and lines put in place for either s.c. or i.v. administration of drug. Laminectomies were performed at the T8 vertebral level and standardized, reproducible, contusive injuries produced by dropping a 10 gram weight 5 cm onto an impounder, the tip of which was resting on the dura of the exposed spinal cord (*Exp. Neurol.* 88:108, 1985). Rats were then connected, by a system that permitted freedom of movement, to variable rate infusion pumps for the allotted times.

Naloxone treated rats (n = 10) were administered a 4 mg/kg bolus at 30 minutes post-injury followed by, at 1 hour post-injury, a 4 mg/kg/hr s.c. infusion of naloxone for 23 hours. Naloxone was dissolved in saline for administration and control rats (n = 9) were administered saline in the same amount and manner.

At 30 min post-injury, TRH treated rats were administered either a 2 mg/kg i.v. bolus followed immediately by a 2 mg/kg/hr i.v. infusion of TRH for 4 hours (a dose previously found to be effective in cats, n = 10) or a 20 mg/kg i.v. bolus followed immediately by a 20 mg/kg/hr i.v. infusion of TRH for 4 hours (a ten-fold higher dose, n = 10). TRH was dissolved in saline for administration and control rats (n = 9) were administered saline in the same amount and manner.

All rats were tested at 1, 7, 14, 21 and 28 days post-injury by a battery of behavioral tests designed to assess both motor and sensory functional deficit (*Exp. Neurol.* 88:123, 1985). Means for inclined plane angle, motor score and combined behavioral score (a cumulative score based on all tests) did not differ significantly in control and experimental groups at any timepoint up to 4 weeks after injury following either naloxone or TRH administration. Possible explanations for lack of effect include differences in species, vertebral level of injury or relative degree and type of injury. (Supported by NIH-NINCDS, NO1-NS-2-2310).

418.5 **LACK OF EFFECT OF METHYLPREDNISOLONE ON FUNCTIONAL DEFICIT AND HISTOPATHOLOGICAL LESION RESULTING FROM CONTUSIVE SPINAL CORD INJURY IN THE RAT.** R. K. Pettegrew, F. Harvey*, R. L. Taylor* and J. R. Wrathall. Department of Anatomy and Cell Biology, Georgetown University, Washington, D.C. 20007.

Steroids are routinely administered to spinal cord injury patients on the basis of animal studies indicating their potential to reduce functional deficit after injury. However, results from other experimental animal studies have been negative and use of methylprednisolone (MP) in patients is currently being examined in a large-scale clinical trial. We have used a recently developed standardized rat model of graded contusive injury (*Exp. Neurol.* 88:108, 1985) to examine the effects of post-injury treatment with MP on resulting functional deficit and histopathological lesion.

Adult male and female Sprague-Dawley rats (200-225 g) were anesthetized and subjected to standardized contusive injuries that produce mild, moderate or severe chronic functional deficits (*Exp. Neurol.* 88:108, 1985). Rats were randomly assigned to treatment or control groups immediately after injury. At 30 min after injury, MP treated rats (n = 28) were given a bolus dose of 100 mg/kg in saline i.p. and a subcutaneous line was put in place to allow infusion of 10 mg/kg/hr for 24 hours using a system that permitted the rats to move freely during the infusion period. The dose was based on maintaining plasma levels in the rat, assessed by HPLC of blood samples, that were similar to those found effective in cats to reduce early detrimental sequelae of spinal cord injury. Controls (n = 30) were administered equivalent volumes of saline in a similar manner.

At 1, 7, 14, 21 and 28 days after injury, functional deficits were assessed by an individual unfamiliar with the injury or treatment group to which individual rats belonged. Behavioral tests included the motor score (a modified Tarlov scale), inclined plane test, and a number of additional tests of reflex and more complex hindlimb function. Results from all tests were used to generate a combined behavioral score (CBS) that we have found to be a sensitive measure of overall deficit after contusive injury (*Exp. Neurol.* 88:123, 1985). At 28 days, the rats were anesthetized and perfused with fixative. Spinal cord tissue centering on the injury site was paraffin-embedded and serial cross-sections prepared for morphometric analysis (*Exp. Neurol.* 88:135, 1985) of lesion area, and areas of residual gray and myelinated white matter at the lesion epicenters (area of maximal damage).

No statistically significant differences ($P \leq 0.05$) were seen in chronic functional deficit as measured by the motor score, inclined plane test or the CBS in comparisons of MP treated and control groups after mild, moderate or severe contusive injury. Nor was there evidence of significant differences between treatment and control groups in morphometric measures of the lesion epicenter.

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418.6 POLYMORPHONUCLEAR (PMN) CELL INFILTRATION IN EXPERIMENTAL SPINAL CORD INJURY. J. Xu*, T. H. Liu*, E. L. Hogan, P. L. Perot Jr.*, C. Y. Hsu. Departments of Neurology and Neurosurgery, Medical University of South Carolina, Charleston, SC 29425.

Post-traumatic necrosis in spinal cord injury is a consequence of a series of progressive pathophysiological events following the initial physical impact. Vascular changes including endothelial damage, platelet aggregation, vasogenic edema, thrombosis, hemorrhage and ischemia play important roles in the secondary injury process. PMNs have been shown to contribute to vascular injury and tissue damage in inflammation and in such disorders as acute lung injury, intestinal and myocardial ischemia by generating free radicals, eicosanoids and active proteases. PMN infiltration is observed in experimental spinal cord injury in histological studies (Balentine JD, 1978; Means ED and Anderson DK, 1983). The present study was undertaken to quantitatively determine the extent of PMN infiltration in an experimental spinal cord injury model in the rat (Hsu CY et al, 1985). Spinal cord injury was induced with a 5 gm brass weight using a modified Allen method. Sham-operated animals received laminectomy only. Following severe injury (100 gm-cm), the injured cord segment was removed and the extent of PMN infiltration assayed by myeloperoxidase (MPO) (Bradley PP, et al, 1982), which is a PMN marker. Standard curves for MPO activity were linear with peritoneal PMN suspensions (1.5 - 100 x 10^3 cells). One unit of MPO activity is equivalent to that from 3.75 x 10^6 cells. Supernatant of cord homogenate in phosphate buffer containing hexadecyltrimethylammonium bromide does not interfere with MPO assay. The sensitivity of MPO assay was 15 x 10^4 cells or 4 x 10^{-3} unit MPO activity per gm wet tissue. No MPO activity was noted in sham-operated cord. MPO activity was detectable as early as 30 min post injury (0.01 ± 0.003 unit/gm wet wt., Mean ± SD, N = 4) and increased progressively to reach a peak at 4 hr. post trauma (2 hr: 0.02 ± 0.007; 4 hr: 4.75 ± 1.07; 6 hr: 2.19 ± 0.56; 18 hr: 2.13 ± 0.27; 24 hr: 1.79 ± 1.06.) Extent of PMN infiltration appeared to be dependent on the magnitude of trauma. Moderate (40 gm-cm) and mild (20 gm-cm) injury resulted in corresponding less MPO activity at 4 hr post trauma (40 gm-cm: 1.77 ± 0.88; 20 gm-cm: 0.91 ± 0.30). Results from these studies indicate an evolving PMN infiltration after trauma peaking at 4 hours and remaining prominent up to 24 hours coincident with evolving post-traumatic necrosis. Further studies will define the exact roles of PMNs in the pathogenesis of post-traumatic vascular injury and tissue necrosis.

418.7 EFFECTS OF TRAUMATIC BRAIN INJURY ON REGIONAL CEREBRAL BLOOD FLOW IN RATS MEASURED WITH MULTIPLE INJECTIONS OF RADIOLABELED MICROSPHERES. I. Yamakami*, T.K. McIntosh and A.I. Faden (SPON: T. Koch). Neurology Service, V. A. Medical Center and Department of Neurology, University of California, San Francisco CA 94143.

The radiolabeled microsphere method was used to evaluate regional cerebral blood flow (rCBF) change following fluid-percussion- (FP) induced traumatic brain injury in rats. Nine rats (450 - 500 g) were anesthetized with isoflurane (1.0%), intubated and artificially ventilated. Cannulae were placed in the left ventricle via the right subclavian artery and in both femoral arteries. For each injection, 100,000 radiolabeled microspheres (15 μ, Cardiovascular Research Institute, University of California, San Francisco) were used, randomly labeled with ^{153}Gd, ^{114}In, ^{57}Co, ^{51}Cr, ^{113}Sn, ^{85}Sr, ^{95}Nb, ^{54}Mn or ^{65}Zn. Microspheres were injected into the left ventricle (1 ml in volume) over 30 seconds; reference blood was withdrawn from the femoral artery at a rate of 0.5 ml/min. In five control uninjured rats, three sequential flow determinations were performed at 1 hour intervals. In four rats, a 2 mm craniotomy was made over the left parietal cortex and moderate parasagittal FP brain injury (2.2 - 2.3 atmospheres) was induced. In the injured rats, three microsphere injections were performed; 15 min prior to injury, 30 min and 2 hours following injury.

In the controls, no significant changes were seen in rCBF, MAP and pCO_2 over time. The rCBF (ml/min/g) calculated from the uninjured rats confirmed data reported by others; lower brainstem = 1.16 ± 0.09; left anterior cortex = 1.72 ± 0.19; left posterior cortex = 1.89 ± 0.21; right anterior cortex = 1.49 ± 0.16; right posterior cortex = 1.87 ± 0.23. Following trauma, there was a profound and significant decline in rCBF over the area of maximal injury (left parietal cortex): from 2.21 ± 0.28 baseline to 0.53 ± 0.03 at 30 min following injury ($p < 0.01$), with slight recovery at 2 hours following injury (0.74 ± 0.13, $p < 0.05$). A small but still significant decrease in rCBF was also observed in adjacent regions ipsilateral to the injury and in the opposite hemisphere as well. These data (1) substantiate the feasibility of multiple radiolabeled microsphere injections to measure rCBF in head injured rats; and (2) demonstrate that there is significant hypoperfusion of the injury region following moderate FP head injury in rats. It is possible that these rCBF changes may contribute to secondary tissue injury after brain trauma.

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418.8 CHANGES IN BRAIN INTRACELLULAR FREE MAGNESIUM INFLUENCE NEUROLOGICAL OUTCOME AFTER EXPERIMENTAL HEAD INJURY. T.K. McIntosh, R. Vink*, I. Yamakami* and A.I. Faden. Neurology Service, V.A. Medical Center and Dept. of Neurology, University of California, San Francisco, CA 94143.

Trauma to the central nervous system (CNS) often results in the activation of delayed or secondary injury mechanisms that develop over a period of hours to days following the initial insult. Recently, using ^{31}P nuclear magnetic resonance spectroscopy (MRS), we have shown that brain intracellular free magnesium [Mg^{2+}] declines following fluid-percussion (FP) brain injury in a manner proportional to the severity of injury (McIntosh *et al.*, JCBF [Suppl.], 1987). The present study further examined the role of intracellular free [Mg^{2+}] in the pathogenesis of delayed CNS injury. In Study I, male rats (450 g) were anesthetized (50 mg/kg sodium pentobarbital, i.p.) and treated 15 min prior to FP brain injury (2.0 atmospheres [atm]) with either $MgSO_4$ (0.15 mEq in 0.25 cc saline, n = 8) (in order to prevent the postinjury fall in intracellular [Mg^{2+}]) or saline (equal volume, n = 8). Saline-treated animals showed a fall in [Mg^{2+}] (from 1.15 to 0.32 mM; $p < 0.05$) by 60 min postinjury as measured by ^{31}P MRS. These animals developed a moderate neurological deficit which persisted up to 4 weeks postinjury. In contrast, animals pretreated with $MgSO_4$ showed no significant change in [Mg^{2+}] following FP injury (from 1.07 to 0.95 mM) as measured by ^{31}P MRS and demonstrated a significant improvement in neurological recovery when compared to saline-treated animals ($p < 0.05$). This improvement in neurological outcome began at 24 h and continued over the 4 week postinjury study period. In Study II, rats (n = 6/treatment) were placed on a Mg^{2+}-deficient or normal diet for 14 days prior to FP brain injury (2.0 atm). Mg^{2+} deficiency produced a 10 - 15% decline in preinjury brain free [Mg^{2+}], as compared to normal animals. Following FP injury, [Mg^{2+}] in normally fed control animals fell from 1.09 to 0.38 mM ($p < 0.05$) by 60 min postinjury. A moderate chronic neurological deficit was observed in control animals, which persisted up to 4 weeks. In hypomagnesic animals, even lower levels of [Mg^{2+}] were observed by 60 min postinjury (from 0.85 to 0.21 mM; $p < 0.05$). Mg^{2+}-deficient animals developed a significantly greater neurological deficit when compared to normally-fed animals ($p < 0.05$); these differences persisted for up to 4 weeks postinjury. Our results suggest that changes in brain intracellular free [Mg^{2+}] may contribute to the delayed damage and neurological deficit that follow traumatic brain injury.

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418.9 ISCHEMIA-INDUCED NEURONAL CELL INJURY: EFFECTS OF THE INNER ESTER DERIVATIVE OF GM1 GANGLIOSIDE. C. Aldinio*, S. Seren*, A. Lazzaro*, N. Schiavo*, G. Vantini, D. Guidolin*, A. Leon and G. Toffano. Fidia Neurobiological Research Laboratories, Abano Terme, Italy.

The systemic administration of monosialoganglioside GM1 has been reported to ameliorate outcome following injury, both mechanical or chemical, to various neuronal systems of adult rodent CNS. These GM1 effects have, in several cases, been shown to be associated with enhanced neuronal cell survival (i.e. cholinergic, dopaminergic or noradrenergic). Furthermore, GM1 or its inner ester derivative (AGF2) have been shown to have beneficial effects in the early postischemic period following transitory cerebral ischemia in cats, rats or gerbils (for a recent review see G. Tettamanti, R.W. Ledeen, K. Sandhoff, Y. Nagai and G. Toffano: "Gangliosides and Neuronal Plasticity", Liviana Press, Padova, Vol.6).

We now report the effect of AGF2 treatment on the delayed hippocampal neuronal damage occurring following severe transitory incomplete ischemia in adult rats (four-vessel occlusion model: Pulsinelli and Brierley, Stroke 10: 267, 1979). In particular, computer-assisted morphometry was used to assess ischemia-induced damage to pyramidal neurons of the CA1 region of the hippocampus of saline or AGF2 treated rats. Only rats which exhibited isoelectric EEG throughout the 30 minute period of occlusion were considered. Saline or AGF2 (5-15 mg/Kg) was first injected intravenously one hour following clipping off and continued intraperitoneally twice a day for 6 days. Rats were sacrificed 18 hours after last injection. Morphometric analysis showed the occurrence of a consistent loss of CA1 pyramidal neurons in the saline-treated rats, an effect which may be partially prevented by the AGF2 treatment. This effect may perhaps be due to reduction in the degenerative events which occur in the acute postischemic phase.

418.10 LEVELS OF EDEMA AND ATPASE ACTIVITY AFTER LOCAL CEREBRAL ISCHEMIA: GM1 GANGLIOSIDE REDUCES EDEMA IN PRIMARY AND PERI-INFARCT AREAS. S.E. Karpiak, D. Hawver*, Y.S. Li*, and S.P. Mahadik. Div. Neuroscience, NYS Psychiatric Inst., Depts. of Psychiatry, and Biochemistry & Molecular Biophysics, Coll. of Physicians & Surgeons, Columbia University, New York, NY 10032.

Our studies of the acute effects of ganglioside injections on CNS injury have shown that GM1 ganglioside treatment reduces edema and associated losses of Na,K-ATPase and intracellular K+ [1]. Recent studies show that after global ischemia in the gerbil, monosialoganglioside (GM1 & AGF2) reduces mortality by 50% and protects levels of cortical and hippocampal Na,K-ATPase activity [2]. We have begun focusing our studies on a rat ischemia model which results in a highly localized cortical infarct [3]. The model involves occlusion of the middle cerebral artery (MCAo) followed by permanent ligation of the ipsilateral common carotid artery (CCAo) together with a 1hr temporary occlusion of the contralateral CCA. The MCAo+CCAo results in a localized cortical parietal infarct area [# 1]. Other cortical regions [#2 (frontal); #3 (occipital); #4 (temporal)] are less affected, representing the peri-infarct zones. At 72 hrs after the MCAo+CCAo we found a greater than 20% decrease in Na,K-ATPase activity in region #1, with no significant decreases in regions #2-4. Mg-ATPase (a marker for mitochondrial function) was decreased by 10% in region #1, and slightly increased in regions #2-4. 72hrs after the MCAo+CCAo we found almost a 4% increase in water content (edema) in the primary infarct region (#1); a 1.8% increase in area #2; a 1.3% increase in area #3; and no change in water content in area #4. Rats treated (i.p.) with GM1 immediately after the MCAo+CCAo (40mg/kg) and 24hrs later (20mg/kg) showed only a 1.4% increase in water in area #1, with no water increases in regions 2,3,4. Acute GM1 treatment significantly reduced levels of edema in the primary infarct area. The elimination of edema in areas 2-4 by GM1 may indicate that ganglioside treatment reduces the extent of ischemia by limiting the peri-infarct zones.

1. Karpiak & Mahadik. J. Neuroscience Research 12:485 (1984).
2. Karpiak, Li, Mahadik. Stroke 18:184 (1987).
3. Chen et al., Stroke 17:738 (1986).

419.11 DOPAMINE AND ISCHEMIC STRIATAL NERVE CELL DEATH
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Striatal dopamine (DA) metabolism increases following transient forebrain ischemia (Harik,S.I. et al.,Neurology 36:971,1986) and has been proposed to be causally linked to ischemic striatal neuronal damage. To test this hypothesis we examined the effects of DA deafferentation and DA receptor modulation on ischemic damage to striatal neurons using a 4-vessel occlusion (4-VO) rat model of transient forebrain ischemia.

Male Wistar rats were treated as follows: Group 1-left intrastriatal infusion of SKF 38393 (5 ug) 15 min. prior to ischemia; Group 2-unilateral left 6-OHDA deafferentation 7 days prior to ischemia; Group 3-unilateral left 6-OHDA deafferentation 7 days prior to ischemia with left striatal injection of SKF 38393 (5 ug) 15 min. prior to ischemia; and Group 4-preischemic injection of vehicle. All rats were subjected to 30 min. of 4-VO and allowed to survive for 3 days. Ischemic striatal damage was graded by light microscopy using the following scale:0=normal, 1=1-10 %,2=11-49%, and 3=> 50% neurons damaged.

	GRADE OF STRIATAL NEURONAL DAMAGE	
	Injected Side	Control Side
SKF 38393 (7)	2.0±0.8	2.0±0.9
6-OHDA (10)	2.4±0.6	2.4±0.5
6-OHDA/SKF (6)	2.6±0.5	2.5±0.6
VEHICLE (5)	2.6±0.4	2.8±0.3

All values expressed as Mean±S.D.
p >0.4 (Kruskal-Wallis Test)

DA deafferentation failed to attenuate striatal neuronal death. Selective D1 receptor activation in either intact or deafferented striata afforded no protection against ischemic damage. Thus,although striatal hypermetabolism occurs after ischemia,these alterations do not appear to significantly contribute to striatal nerve cell death.

419.12 MPP+ INCREASES EXTRACELLULAR POTASSIUM IN RAT STRIATAL SLICES: PRELIMINARY EVIDENCE THAT CONSEQUENCES OF MPP+ NEUROTOXICITY ARE SPREAD BEYOND DOPAMINERGIC TERMINALS.
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MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) produces a clinical syndrome similar to idiopathic Parkinson's disease in primates. The active metabolite of MPTP, MPP^+ (1-methyl-4-phenylpyridinium), has been shown to selectively destroy nigrostriatal dopaminergic (DA) neurons. Previous studies indicated that MPP^+ was actively accumulated by DA terminals and that MPP^+ inhibited electron transport, and thereby the oxidative production of ATP, in mitochondria. Consistent with this suggestion, MPP^+ increased oxidation of cytochrome b in slices of rat striatum but not in hippocampus where there were very few DA terminals. The effect of MPP^+ in striatum was antagonized by the specific dopamine uptake inhibitor, mazindol. The amplitude of cytochrome b oxidation, however, was greater than that expected from a selective effect of MPP^+ on DA terminals since these do not likely comprise more than 2% of the total striatal mass. Such studies led to the hypothesis that the effects of MPP^+-neurotoxicity were not confined to DA terminals but were apparent in neighboring cells with consequences upon energy requiring activities such as ion transport. To test this hypothesis, extracellular potassium ion activity (K^+o) was measured by ion selective microelectrodes in slices of striatum and hippocampus isolated from rats anesthetized with ether. These slices were housed in chambers designed for spectrophotometric and electrode monitoring and bathed in warmed, oxygenated artificial cerebrospinal fluid. MPP^+ addition (1 um) to striatal slices elevated K^+o from its baseline activity of approximately 3.5 mM to over 12 mM over a 2 hour period. This MPP^+ effect was antagonized by mazindol and did not occur in hippocampal slices, confirming that MPP^+ influences require the selective uptake of MPP^+ by DA terminals. However, the large K^+ efflux suggests that cells other than DA terminals were influenced by MPP^+ secondarily to primary effects of MPP^+ in DA terminals. Two hypotheses are proposed: that energy failure in DA terminals results in leakage of neurotoxic substances or metabolites altering membrane conductance properties of adjacent cells and thereby placing additional demand upon ion transport pumps and mitochondrial oxidative phosphorylation; or that there is secondary uptake of MPP^+ leading to mitochondrial inhibition in cells neighboring DA terminals. Whatever the mechanism, these data indicate that actions of MPP^+ require the presence of functioning DA terminals but consequences of MPP^+-neurotoxicity are not ultimately confined to these terminals. (Supported by the National Parkinson Foundation, PHS grants NS 14325 and HL38657 and the American Heart Association).

419.13 THE TOXICITY OF MPTP ON CULTURED NEUROBLASTOMA CELLS IS MEDIATED BY MPP^+.
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1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine (MPTP) has been shown to induce symptoms of Parkinson's disease in both humans (Langston et al., Science 219:979, 1983) and primates. MPTP is rapidly oxidized in the brain to 1-methyl-4-phenylpyridium ion (MPP^+), via monoamine oxidase (MAO). The conversion of MPTP to MPP^+ is mediated mainly by glial cells (e.g. Ransom et al., Soc. Neurosci. Abstr. 12:83, 1986). Neuronal toxicity appears to result when MPP^+ enters the intracellular compartment. Experiments reported here provide additional evidence that MPP^+ (and glial cells) play an important role in the toxic effects of MPTP on neurons.

Neuroblastoma cells (NG108) and astrocytes (prepared from one day old mouse forebrains) were grown in cell culture. Varying concentrations of MPTP (1-100 µg/ml) or MPP^+ (0.1-10 µg/ml), or astrocyte conditioned medium were applied for five days to neuroblastoma cultures. Astrocyte conditioned medium was produced by exposing astrocyte cultures to MPTP (25-50 µg/ml) for 24 hrs. The effect of various treatments on neuroblastoma density was determined by direct cell counts. In some experiments MPP^+ was quantitatively assayed using gas chromatography-mass spectrometry.

MPTP, at high concentrations, produced a mild decrease in neuroblastoma cell density which was blocked by co-incubation with the MAO inhibitor pargyline. Neuroblastoma cell density was markedly reduced by astrocyte conditioned medium containing concentrations of MPTP which generally had only minor effects on cell density when applied directly to neuroblastoma cultures. The effect of astrocyte conditioned medium was not blocked by pargyline. MPP^+ applied to pure neuroblastoma cultures diminished cell density in a dose-dependent manner at concentrations above 1 µg/ml. Direct measurements of MPP^+ indicated that astrocyte cultures contained concentrations of MPP^+ in the range of 5-10 µg/ml after incubation with MPTP (25 µg/ml) for 24 hrs.

The toxic effect of MPTP on cultured neuroblastoma cells appears to be mediated by MPP^+. Thus, the enhanced toxicity of MPTP solutions after preincubation in the presence of astrocytes, is probably the result of more rapid conversion of MPTP to MPP^+ by astrocytes than by neuroblastoma alone. Some conversion of MPTP to MPP^+ by neuroblastoma cells is expected, however, since these cells are known to contain variable amounts of MAO. Additional experiments are in progress to directly measure the rate at which neuroblastoma cells convert MPTP to MPP^+.

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419.14 2'-CH3-MPTP: A MORE POTENT DOPAMINERGIC NEUROTOXIN THAN MPTP IN PRIMATES?
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Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to primates has been shown to induce a parkinson-like syndrome with neurochemical, behavioral and neuropathological abnormalities. A number of analogues of MPTP (over 30) have been synthesized and tested in an attempt to discover the structural features of MPTP-like compounds that are important in the development of neurotoxicity. One such analogue 1-methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydropyridine (2'-CH3-MPTP) has been reported to be even more toxic than MPTP when administered to C-57 mice, causing very large decrements in neostriatal DA, HVA, DOPAC; severe aphagia, adipsia, akinesia, tremor; and complete loss of neurons in the zona compacta of the substantia nigra (Youngster et al., Eur J Pharm, 122, 1986).

In the present study we describe our experience with 2'-CH3-MPTP in the primate (Macaca fascicularis). Primates were treated with 2'-CH3-MPTP (N=3), MPTP (N=3) or isotonic saline (N=3) for three consecutive days (0.35 mg/kg, iv). Lumbar CSF was sampled once prior to initiation of treatment and again 4 and 11 days after the last treatment day. CSF was collected on dry ice via lumbar puncture in 3 sequential aliquots (1.5 ml each). Animals were sacrificed by pentobarbital overdose (40 mg/kg, iv) 12 days after the last MPTP injection. The brain was removed from the cranial cavity, rinsed in ice cold saline, cut into 5 mm thick coronal sections which were immediately frozen on stainless steel trays on dry ice within 6 min of death. Samples of SN were taken before freezing and fixed for histological examination. Levels of biogenic amines were determined utilizing HPLC procedures with electrochemical detection. CSF studies revealed a significant reduction in the levels of norepinephrine and HVA 4 days post-treatment in MPTP and 2'-CH3-MPTP groups. By 11 days post-treatment, NE levels returned to baseline whereas HVA levels showed even greater reductions. Nigral cell counts in MPTP and 2'-CH3-MPTP groups were 65% and 53%, respectively, of controls. Striatal dopamine levels were 16% (MPTP group) and 13% (2'-CH3-MPTP group) of control striatum. Behaviorally, the 2'-CH3-MPTP monkeys exhibited a more severe Parkinsonian symptomology than the MPTP treated primates, with a behavioral rating of 3.7 compared to 2.3 for MPTP treated animals. The data of the present study indicate that 2'-CH3-MPTP is neurotoxic in primates as is MPTP. Further work will be required to determine if there are any differences, subtle or major, between the two neurotoxicants. (Supported by Sigma Tau Pharmaceuticals and the Veterans Administration.)

418.15 SOMATOFUGAL AXONAL ATROPHY PRECEDES AXONAL DEGENERATION IN ACRYLAMIDE NEUROPATHY. J.W. Griffin[2], B.G. Gold[1] and D.L. Price[2] [1]Rutgers University College of Pharmacy, Piscataway, NJ 08854 and [2]The Johns Hopkins University School of Medicine, Baltimore, MD 21239

Somatofugal axonal atrophy is a component of the axon reaction produced by axotomy. Acrylamide (AC), which produced a distal axonopathy upon repeated exposures, also results in the development of axonal atrophy in the proximal portion of fibers (Gold, Griffin and Price, J. Neurosci., 1985). The mechanism by which the axon reaction is initiated remains unknown, although it presumably involves an alteration in delivery of a "trophic" signal to the perikaryon. In the present study, we have asked whether AC, which interfers with retrograde transport following a single injection (Miller and Spencer, J. Neurochem., 1984), can initiate this perikaryal response prior to axonal degeneration.

Three 5-week old rats were given a single high dose of AC (75mg/kg) followed by 4 daily intraperitoneal injections (30mg/kg). Twenty-four hours after the last injection (i.e. 5 days following the first injection) the animals, along with age-matched controls, were perfused with 5% glutaraldehyde. Dorsal root ganglia (DRG) and dorsal roots were removed, cut into 5 consecutive 2mm segment, and prepared for light microscopy. Thick sections (1um) from the center of each block, corresponding to 1,3,5 and 7mm from the edge of the DRG, were stained with toluidine blue. Morphometric analysis was performed on light microscopic prints (800 fibers/section) to determine axonal diameter, area, perimeter and myelin sheath thickness (MST). Shape factor, and indicator of deviation from circularity, was also calculated. Sciatic nerve branches and muscles of the hindfeet were also examined by light microscopy.

Dorsal root fibers appeared less circular in shape at the DRG and 1 and 3mm from the DRG. Histogram constructed from axonal diameters showed a significant ($p<.05$; Mann-Whitney U-test) shift to smaller caliber at these levels; the distribution of calibers were unaltered from controls both 5 and 7mm from the DRG. Mean values were significantly ($p<.05$; repeated measures ANOVA) reduced up to 3mm from the DRG. No degeneration was present. Additional studies showed that the atrophy progresses distally with time.

Somatofugally spreading axonal atrophy precedes the development of axonal degeneration in AC neuropathy. Moreover, the results suggest that the response begins earlier than the 5 day time-point chosen for study. It is suggested that at least some of the perikaryal changes observed in AC neuropathy may reflect a secondary response to axonal injury even if they arise prior to axonal degeneration, and that altered "trophic" functions may play a role in the pathogenesis of AC neuropathy.

418.16 IDENTIFICATION OF A NOVEL PROTEIN INDUCED IN THE SYMPATHETIC NERVOUS SYSTEM FOLLOWING GUANETHIDINE TREATMENT. P. Manning, R. Leimgruber*, J. Radosevich* and D. Tiemeier*. Monsanto Co., St. Louis, MO 63198.

Guanethidine, an adrenergic neuron blocking agent, produces marked and permanent destruction of the sympathetic nervous system of rats, hamsters and monkeys when administered chronically in high doses. Neuronal destruction appears to occur by a cell-mediated autoimmune mechanism. It can be completely prevented by concurrent treatment with nerve growth factor (NGF), possibly by affecting expression of an antigen(s) which induces the immune response. Experiments were conducted to identify novel proteins (potential antigens) induced by guanethidine and whether NGF affects their expression. One-week-old rats and mice were treated daily with 50 mg/kg of guanethidine sulfate for various lengths of time alone or concurrently with 5 mg/kg of NGF. Homogenates of control and treated superior cervical ganglia (SCGs) were analyzed by 2-dimensional gel electrophoresis and the protein patterns were compared. A novel protein of approximately 70,000 daltons was detected three days following the start of treatment, a time prior to lymphocytic infiltration. It increased in intensity during the period neuronal cell death occurred and decreased in intensity thereafter. In animals treated with NGF concurrently histological protection from guanethidine treatment was observed and the novel protein was not detected. Also, this protein was not present following treatment with non-cytotoxic doses of guanethidine, nor was it present in ganglia obtained from untreated rats. The protein, which we have named GIP-70 (guanethidine-induced protein), has a pI of 6.9 in urea. To eliminate the possibility that GIP-70 is a product of activated, invading lymphocytes, rats were pretreated with cyclophosphamide, an immunosuppressive agent which blocks tissue destruction and lymphocytic infiltration into the ganglia, but not accumulation of guanethidine within neurons. GIP-70 was detected in extracts from these ganglia, suggesting it is of ganglionic origin. Rats were also treated with either 6-OHDA or antibodies to NGF, both of which cause destruction of sympathetic neurons by non-immunological mechanisms. GIP-70 was not detected in ganglia from these rats suggesting that it is not a protein generally related to neuronal cell death. Our previous experiments have shown that mice do not exhibit neuronal destruction following guanethidine treatment. A protein analogous to GIP-70 also was not present in treated ganglia from mice. This data is consistent with GIP-70 being an antigen involved in guanethidine-induced, autoimmune sympathectomy and with NGF providing a protective role in autoimmune responses.

MOTOR SYSTEMS

419.1 DEVELOPMENTAL PHARMACOLOGY OF LOCOMOTION IN SPINAL AND INTACT TADPOLES AND FROGS. D.J. Stehouwer. Department of Psychology and the Center for Neurobiological Sciences, Univ. Florida, Gainesville, FL 32611

Electrophysiological recordings from the isolated nervous system of the tadpole reveal phase-locking between bursts of motoneurons innervating the tail and hindlimbs during fictive locomotion, suggesting that the pattern generators that produce alternating undulations of the tail of the tadpole may also serve to coordinate stepping. However, cervical spinal transection abolishes tail-beating in tadpoles while greatly augmenting hindlimb stepping in the same animals, suggesting that coordinated movements of the tail and limbs are not produced by the same spinal circuits. The experiments presented here were conducted to determine whether or not circuits underlying undulatory swimming and stepping can be pharmacologically dissociated.

To examine this question, tadpoles and newly metamorphosed frogs were injected intraperitoneally with a variety of drugs that have been shown to elicit locomotion in other vertebrates: l-dopa; amphetamine; apomorphine; n-methyl-d-aspartate (NMDA); glutamate; and kainate. The effect of the convulsants strychnine and picrotoxin were also examined. Of these drugs, only NMDA and picrotoxin proved to have any effect on locomotion. Dose response curves for these drugs were established and their relative effectiveness compared in tadpoles and froglets that were intact or had received complete medullospinal transections. Picrotoxin produced a four-fold increase in locomotion of intact tadpoles but had no effect on locomotion of spinal tadpoles. Conversely, NMDA had no effect on the level of locomotion of intact tadpoles but produced high levels of locomotion in the normally inactive, spinally transected tadpoles. However, this swimming was not accompanied by movements of the developing hindlimbs, despite the fact that spinal transection augments hindlimb stepping at those stages of development. Neither picrotoxin nor NMDA increased locomotion of intact froglets. Picrotoxin had no discernible effect on froglets at subconvulsive doses, but NMDA produced sudden extension of the hindlimbs in spinal subjects, which occurred in single hindlimbs and synchronously in both hindlimbs, but never in repeated alternation between the hindlimbs.

These results do not support the suggestion that shared spinal circuits coordinate stepping and alternating contractions of axial muscles of developing frogs.

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419.2 DEVELOPMENT OF LOCOMOTION IN HAMSTERS WITH NEONATAL LESIONS OF THE PYRAMIDAL TRACT. J. Keifer and K. Kalil. Neurosciences Training Program and Dept. of Anatomy, University of Wisconsin, Madison, WI 53706

There is considerable evidence that injury to the CNS in infant animals may result in dramatic anatomical and functional reorganization of neural circuitry. However, preservation of normal motor function attributable to specific anatomical rearrangements has been difficult to demonstrate. Therefore, the present study was undertaken to correlate the regrowth of pyramidal tract (PT) fibers which occurs after infant lesions of this pathway with the development of locomotion to determine whether the establishment of PT terminations in the spinal cord is correlated with the maturation of intralimb coordination.

Locomotion of normal infant hamsters and those with a PT lesion at postnatal day 5 was filmed during development until 4 weeks of age. A joint angle analysis of the position of the forelimb and hindlimb during the step cycle was performed to quantify the development of limb excursion during stepping.

During normal locomotion, infant hamsters show an excessive angular excursion of all the joints of the forelimb and hindlimb which is gradually reduced to adult levels by 16 to 18 days. Infants given a PT lesion express abnormal joint excursions such that both limbs are hyperextended as compared to normal infants which results in a delay in the appearance of normal limb excursion to shortly after 18 days.

Locomotion of infant lesioned animals was also compared with those lesioned as adults. The primary deficit found in adult lesioned hamsters is a marked reduction of the yield phase of the step cycle of the forelimb and hindlimb, which did not recover. In contrast, infant lesioned hamsters develop a normal yielding phase of the hindlimb; however, the forelimb yield fails to develop normally.

These results suggest that during the normal development of locomotion the PT may play a role in the maturation of limb excursion. Hyperextension of the limbs after early lesions may be due to a delay in the innervation of the spinal cord by PT fibers which provide descending inhibition as previously reported (Soc. Neurosci. Abs. 12: 880, 1986). When infant lesioned animals mature, development of normal locomotor function is correlated with the regrowth of severed PT axons to their appropriate sites of termination in the cervical and lumbar spinal cord. However, forelimb movements remain permanently impaired. This suggests that the control of the forelimb requires a precision of neural input for which regrowing PT fibers, or other descending pathways, cannot compensate.

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419.3 Mechanisms of Motor Activity in the Isolated Lumbar Cord of the Chick Embryo Investigated by Ventral Root Current Injection and Inhibitory Blockade. Michael J. O'Donovan and M.A. Barry*. Department of Physiology and Biophysics, University of Iowa, Iowa 52242.

The goal of the present experiments was to establish the influence of motoneuron membrane properties and synaptic inhibition on the pattern of motor activity generated by the isolated spinal cord of 6-8 day (st.26-32) chick embryos. Electrotonic recordings from ventral roots and muscle nerves were used to monitor motoneuron excitatory drive. A new technique - ventral root current injection - was used to polarize the membranes of a population of motoneurons to examine their excitability and the voltage dependence of their synaptic potentials. The use of a bridge circuit enabled current to be injected through the ventral root electrode while simultaneously recording the motoneuron depolarization. The ability of current injected through the ventral roots to polarize motoneuron membranes was verified in older embryos using simultaneous intracellular recording.

The electrotonic recordings revealed that motoneurons were depolarized during each cycle of activity but discharge was largely restricted to the early part of the depolarization. In the youngest animals (st.26-28) the depolarization was briefer than at other stages. The amplitude of the depolarization was increased by hyperpolarizing current and reduced by depolarizing current injected into the ventral roots. Prolonged membrane hyperpolarization and sudden depolarizing current steps did not block or trigger motoneuron activity. Similar findings were obtained in older embryos using intracellular recording and suggest that the depolarization is the product of chemical synaptic action and not an intrinsic property of motoneurons. Stage 30 motoneurons were also capable of maintained discharge in response to depolarizing current injection, which suggests that the absence of late motoneuron discharge during prolonged synaptic depolarization is not simply due to an immaturity of repetitive firing mechanisms. Bath application of inhibitory blockers (picrotoxin 10^{-4}M; Strychnine 10^{-5} M) reduced the rate of spontaneous motor activity and increased both the amplitude of the depolarization and the intensity of motoneuron discharge. The results indicate that motor activity is produced by similar synaptic mechanisms in young and old embryos and that synaptic inhibition is involved in the genesis of motor activity very early in development. Supported by NSF BNS 8402838.

419.4 A KINEMATIC STUDY OF CHICK EMBRYONIC MOTILITY. S.J. Watson* and A. Bekoff (SPON: S.M. Royer). Dept. EPO Biology, University of Colorado, Boulder, CO 80309.

Spontaneous motility of the chick embryo *in ovo* has been described from behavioral observations as consisting of periodic sequences of random and jerky movements of the trunk, neck and limbs (Hamburger, V., *Quart. Rev. Biol.*, 38:342, 1963). Hamburger reported that there was no coordination between body parts, such as the two legs, and no information was provided on the nature of coordination within a limb. However, EMG analysis of leg muscles has demonstrated a high degree of coordination of ankle muscle activity by 7 days of incubation (Bekoff, A., *et al., P.N.A.S.*, 72:1245, 1975), and coordination between knee and ankle synergistic muscles by 9 days of incubation (Bekoff, A.,*Brain Res.*, 106:271, 1976). Hence, EMG data support the view that the neuronal circuitry underlying intralimb coordination is organized at early stages in development even though the behavior appears random and uncoordinated to the observer. Using kinematic methods, we sought to obtain a more quantitative description of embryonic leg movements and to determine whether there is a consistent pattern of coordination between limb segments.

A window was made in the shell of fertilized eggs on embryonic day 9 or 10 and the extraembryonic membranes were deflected to reveal the embryo positioned on its left side. The lateral aspects of the sacrum, hip, knee and ankle of the right leg of the embryo were marked with a dot of black paint. Spontaneous movements of the leg were video recorded at 16.7 frames per second and a computer program was used to digitize joint angles frame-by-frame. Cycles of flexion and extension that produced relatively large excursions of the leg were chosen for analysis.

For the leg movements analyzed so far, cycle period varied from 170 to 2000 ms. Typically, the knee and ankle joints flexed and extended synchronously. Motion at the hip could be synchronously paired with corresponding motions at the distal joints, synchronously paired with the reverse motions at the distal joints, or show very little angle change during the cycle.

These data demonstrate that movement of an individual leg during embryonic motility is not random. Rather, joint motions can be coupled in specific patterns of coordination. Furthermore, these results are not only consistent with the results of the EMG studies referenced above, but demonstrate that the organized motoneuronal output is reflected in the resulting leg movements. Supported by NIH grant NS 20310.

419.5 EMERGENCE OF FLEXION AND EXTENSION MUSCLE SYNERGIES IN THE HINDLIMB OF CHICK EMBRYOS. N.S. Bradley and A. Bekoff, Dept. EPO Biology, University of Colorado, Boulder, CO 80309.

EMG recordings of motility in intact chick embryos (Bekoff, A., *PNAS*, 72:1245, 1975) and isolated spinal cord-hindlimb preparations (Landmesser, L.T., and M.J. O'Donovan, *J. Physiol.*, 347:189, 1984) indicate intralimb neuromuscular patterns are present at 7 to 10 embryonic days. It has been proposed that the patterns emerge sequentially: 1st, muscles with synergistic or antagonistic action at a joint are synchronously or reciprocally activated, respectively; 2nd, muscles with synergistic actions at different joints are synchronously activated; 3rd, these patterns are established within a limb before they are coordinated between limbs (Bekoff, A., *Brain Res.*, 106:271, 1976). Our goal, therefore, is to determine whether this sequence characterizes development of embryonic motility in chicks.

At 6 to 10 embryonic days, selected hindlimb muscles of White Leghorn embryos are exposed by dissection for EMG recordings: gastrocnemius lateralis (GL, ankle extensor), tibialis anterior (TA, ankle flexor), caudilioflexorius (CF, hip extensor-knee flexor), and sartorius (SA, hip flexor-knee extensor). Suction electrodes are placed on the surface of each muscle to obtain 4-channel EMG recordings of spontaneous motility *in ovo*. To characterize the emerging patterns, cycle period, the time between onset of successive bursts in the reference muscle (GL), phase relationships of non-reference muscles, and burst durations for all muscles are measured.

To date, 4-channel EMG recordings in chicks at 10 embryonic days confirm the presence of the neuromuscular patterns reported by Bekoff '75 and Landmesser and O'Donovan '84, referenced above. Further, our records indicate that the extensor (GL and CF) and flexor (TA and SA) synergies are present and reciprocally active. Effort is currently underway to determine whether flexors and extensors are similarly coupled and reciprocally active in intact chick embryos at earlier ages.

It has been proposed that unit CPGs (central pattern generators) control reciprocal motions for each joint within a limb and together control rhythmic limb movements. These units may be separately modulated during certain ballistic movements to produce mixed synergies (Smith, J.L., *et al., J. Neurophysiol.*, 54:1271, 1985). However, in chicks, there is only limited modulation of flexor and extensor synergies during non-ballistic movements suggesting that unit CPGs may not be readily uncoupled in the intact animal. Determining if neuromuscular patterns emerge sequentially may clarify whether unit CPGs are fundamental controlling networks, and to what extent CPGs are initially uncoupled and become coupled during development. Supported by NIH grant NS 20310.

419.6 ORGANIZATION OF VESTIBULO-OCULAR PROJECTIONS IN THE CHICKEN EMBRYO. G. Petursdottir* and J.C. Glover. Institute of Physiology, University of Oslo, 0162 Oslo 1, Norway

We are studying the organization and development of the vestibular projections of the chicken embryo. Using retrograde tracing techniques, we demonstrated previously that vestibulo-spinal neurons can be segregated into 3 discrete, coherent groups that project to the spinal cord by different pathways (Glover et al., 1986, Soc. Neurosci. Abst. 12: 1119). This spatial segregation is apparent at least as early as the time that the vestibulospinal axons first reach the spinal cord. Since the vestibular nuclei are also the source of a major ascending projection to oculomotor nuclei, we have performed further retrograde tracing experiments to determine the organization of vestibulo-ocular neurons and their spatial relationship to the vestibulospinal groups.

An in vitro preparation of the brainstem was used to allow for the precise, delineated application of the retrograde tracers HRP, fluorescein-dextran-amine, and rhodamine-dextran-amine. The latter two tracers were used in double labelling experiments to determine whether single vestibular neurons project to multiple targets or along multiple pathways. To label the vestibulo-ocular projection the tracers were injected just caudal to the trochlear nucleus.

The vestibulo-ocular projection arises from a large population of vestibular neurons, extending both rostral and caudal to and partially overlapping the vestibulospinal groups. In the region of overlap there is extensive intermingling of vestibulo-ocular and vestibulospinal neurons, but very few (in many preparations no) vestibular neurons project to both targets. Thus, the vestibulo-ocular and vestibulospinal populations are almost completely distinct, a situation that exists even at the earliest times that these neurons can be retrogradely labelled.

Vestibulo-ocular axons project directly towards the midline and then make an orthogonal turn to ascend in the MLF. The vestibulo-ocular neurons projecting in the ipsilateral and contralateral MLF are clustered and exhibit well-defined spatial segregation, with limited intermingling. Thus, like the vestibulospinal neurons, the vestibulo-ocular neurons are organized into discrete, coherent groups on the basis of their projection pathway. Orthograde tracing is being attempted to determine how early this organization is expressed.

419.7 TRANSPLANTS ENHANCE RECOVERY OF LOCOMOTOR FUNCTION AFTER SPINAL CORD LESIONS IN NEWBORN RATS. E. Kunkel-Bagden* and B.S. Bregman. Dept. Anatomy, Univ. MD Sch. Med., Baltimore, MD 21201

We have demonstrated previously that fetal spinal cord transplants support neuronal survival and axonal elongation in the injured immature spinal cord. The current experiments were designed to determine whether these transplants also promote recovery of motor function following neonatal spinal cord lesions. A total of 18 animals were used in this study. A spinal cord hemisection was made at a mid-thoracic level in 12 rat pups (<24 hrs). In half of these animals a transplant of fetal spinal cord tissue (E14) was placed into the lesion site (HX+TP), while the others received no transplant (HX). Six littermates served as controls (CON). The animals were examined qualitatively on a battery of reflex and locomotor tests during development (1-28 days postnatal) and as adults (1-6 months). Quantitative analysis of locomotion was obtained at 4-6 mo. on a treadmill (footprint analysis), on a grid runway (50mm squares), and on videotape.

HX, HX+TP, and CON animals were able to traverse a solid runway with little difficulty. On the grid runway, CON animals also crossed easily by placing the paws onto the supports, making few errors in foot placement (<3 errors/100 steps). HX and HX+TP animals made a significant number of errors both ipsilateral and contralateral to the lesion (HX = 18 errors/100 steps, HX+TP = 15 errors/100 steps, ipsilateral). The groups differed, however, in their response following an error. HX+TP animals immediately replaced the limb onto the grid, whereas the HX animals flexed and extended the limb repeatedly prior to replacing the limb for support. Footprint analysis of treadmill locomotion revealed quantitative alterations in locomotor parameters in both HX and HX+TP groups when compared with the CON group. The base of support as measured by the distance between the feet was the same in the CON, HX, and HX+TP animals. HX results in an increased lateral rotation of the hindlimbs when compared with CON. The amount of limb rotation in HX+TP animals was more similar to that observed in control animals (CON=17±1.6°, HX+TP= 25±1.7°, HX=38±1.6°). Similarly, step length was significantly decreased by the hemisection, whereas the presence of a transplant enabled the animals to use a locomotor step pattern similar to that in CON animals (CON=67±5mm, HX+TP=56±2mm, HX=34±5mm). None of the characteristics of locomotion that we have examined returned to levels identical with control values. However, animals that received the transplants demonstrated qualitative and quantitative improvements in several parameters of locomotion. This alteration indicates that neural tissue transplants are able to mediate recovery of motor function after spinal cord hemisection.

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419.8 ACTIVITY OF PYRAMIDAL TRACT NEURONS IN FETAL OCCIPITAL CORTEX TRANSPLANTED TO ROSTRAL CORTEX. L.L.Porter, J.M.Cedarbaum, D.D.M.O'Leary, B.B.Stanfield, and H.Asanuma. Neurophysiology, The Rockefeller Univ.,New York, NY 10021, Dept. Neurosurg.,Washington Univ.Sch.Med.,St.Louis, MO 63110 and NIMH, Poolesville, MD 20837.

During early postnatal development in the rat, cells in layer V of occipital as well as rostral cortex extend pyramidal tract axons. All of the occipital pyramidal tract axons are eliminated by the end of the third postnatal week, although their cells of origin do not die. When a piece of fetal occipital cortex is transplanted to a rostral cortical locale in newborn pups, some of the transplanted occipital neurons extend a pyramidal tract axon which they maintain long after they would have been eliminated had they remained in their occipital locale (Stanfield & O'Leary, Nature,1985). We now report that such pyramidal tract axons are capable of transmitting signals and thus may carry functionally relevant messages.

Pieces of occipital cortex were taken from fetuses of a female which 2 days before had been injected with ^{3}H-Thymidine. Months later the host rats were anesthetized, stabilized and a recording/stimulating microelectrode was aimed at the transplant. In some cases a stimulating electrode was stereotaxically placed in the lower pyramidal tract. In general, the level of unit activiy within the transplants was less than in the adjacent host cortex. Although we could not reliably evoke movement with low level microstimulation within a transplant, some cells within the transplants responded to pyramidal tract stimulation with short latency antidromic spikes. Tests for latency shifts with changes in current intensity and for duration of refractory periods were made to accurately determine that responses were antidromic. Current was passed through the recording electrode to make a small lesion at sites of antidromically activated cells. The boundaries of the transplants were subsequently determined autoradiographically on frozen sections through the brains. In 2 cases the marking lesions were clearly well within the transplant. Our results confirm that fetal occipital neurons transplanted to the rostral cortex can extend and maintain pyramidal tract axons and furthermore, that these axons are capable of transmitting impulses. Although microstimulation of the transplant could not evoke movement, this may be explained by the fact that within the transplants pyramidal tract neurons are present in relatively low density and are not tightly packed within a narrow layer V as they are in normal cortex. It thus remains uncertain if these axons form normal synaptic connections or carry functional information.

419.9 POSTNATAL MATURATION OF LATERAL GASTROCNEMIUS MOTOR UNITS. M.K. Cook*, J. Bussey*, S.P. Donahue*, and A.W. English, Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322,SPON: R. Fricke

Architectural and morphometric analyses of the rat lateral gastrocnemius (LG) indicate that LG muscle fiber number doubles by the fourth postnatal (P4) day and reaches the adult size by P8 in each of its three unipennate heads. This increase is due to the rapid differentiation of secondary myotubes. In light of the evidence that polyinnervated fibers are found in LG until P16 (Bennett and Lavidis, J. Neurosci. 4:2204-2212, 1984), we studied the physiological properties of individual LG motor units in neonatal rats to examine what role synapse elimination might play in their maturation. Animals were anesthetized using hypothermia. The triceps surae muscles, the sciatic nerve, and the intact L4 and L5 ventral roots were then removed. Motor units were isolated IN VITRO by splitting the rootlets until a single active LG axon was present. The contractile properties of the unit were examined with supramaximal stimulation of the rootlet and evoked electromyographic (EMG) activity was recorded from up to 12 different bipolar recording sites on the ventral surface of the muscle using a flexible circuit electrode to localize the muscle fibers innervated. All motor units from rats aged <P4 were slow contracting and a substantial proportion (50%) responded to repeated tetanic stimulation as highly or intermediately fatiguable (fatigue index <0.5). A bimodal distribution of large and small units was encountered. By P8, nearly all units had attained their adult level of relative force production but contraction speeds were still quite slow. By P16, the properties of all motor units examined were essentially as noted in adult rats. No significant differences were found between L4 and L5 motor units. At all ages, evoked EMG activity indicated that individual motor unit territories were highly compartmentalized. Reduction in motor unit size occurs over the same time course in all four neuromuscular compartments and for L4 as well as L5 units. Such reduction is completed before P4 for some motor units and for all units by P8. Presumably, a selective addition of secondary myotubes occurs at about the same rate that synapses are being eliminated from polyinnervated primary myotubes. Supported by NS20545 from the USPHS.

419.10 POSTNATAL DEVELOPMENT OF COMPARTMENT NUCLEI OF CAT LATERAL GASTROCNEMIUS. A.W. English and M.K. Cook*, Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322

A compartment nucleus is the collection of motoneurons which innervates a neuromuscular compartment. In adult animals compartment nuclei (CN) are arranged in a topological manner in the spinal cord (Weeks and English, J. Comp. Neurol. 235:255-267, 1985). Reports of motoneuron cell death in the early postnatal period and the suggestion that the axons of neonatal motoneurons might have multiple long branches prompted us to investigate CN arrangement in the spinal cord of kittens. Kittens ranging in age from postnatal day six (P6) to P32 were anesthetized with ketamine HCl. On one side of the animal a single primary branch of the nerve to lateral gastrocnemius and soleus (LG-S) was cut and soaked in a solution of horseradish peroxidase (HRP) for 1-2 hours. Each primary branch of the LG-S nerve is known to innervate a single neuromuscular compartment in adults. On the other side of the animal, the entire LG-S nerve was treated similarly. After a 48 hour survival period, the animals were sacrificed and the L6-S2 spinal cord segments sectioned and reacted for demonstration of HRP. The number of labelled LG-S motoneurons in kittens is not significantly different from that of adult cats, which indicates that no significant postnatal cell death is found in this motor nucleus. The number of motoneurons labelled after soaking different primary muscle nerve branches is also not significantly different from the number found after soaking the same branch in adult cats. Thus, no extensive pruning of long axon branches to different compartments is found. In all instances a bimodal distribution of motoneuron sizes was found. The proportions of these alpha and gamma motoneurons in each CN were similar to adults. The different CN are topologically organized but are arranged in a non-isometrically scaled down version of the arrangement seen in cats. All spatial parameters are relatively smaller in the kittens. Thus the CN of LG might be compact and spatially distinct masses which could be used as targets for afferent systems forming synapses in the neonatal period. Supported by NS20545 from the USPHS.

419.11 ACTIVITY DEPENDENT REGULATION OF ACETYLCHOLINE RECEPTORS IN DEVELOPING NORMAL AND MED MOUSE MUSCLE. Joanne M. Yeakley* and C. Gary Reiness. Dept. of Biology, Pomona College, Claremont, CA 91711

We have investigated whether muscle activity is necessary for the postnatal loss of extrajunctional acetylcholine receptors (AChRs) that normally occurs in rodent muscle. To do this, we have used mice with motor endplate disease (med mice), a hereditary recessive disorder that causes failure of neuronal conduction of action potentials, and thus loss of muscle activity, at about 10d after birth, during the period of postnatal decline of extrajunctional AChRs.

We examined AChRs in biceps brachii (long head) and rectus femoris muscles, which are severely affected by the med mutation, in med mice and in normal littermates between 5 and 21d after birth. In normal mice, AChR levels, determined by ^{125}I-α-bungarotoxin binding to extracts, declined from about 10 fmol AChR/mg of tissue wet weight at 5d to about 2 fmol/mg at 21d. In med littermates, AChR levels followed the normal time course until about 10d, then rose to about 20 fmol/mg (biceps) or 12 fmol/mg (rectus) by 21d. We established that at least 80% of the AChR in these muscles is surface, not internal, AChR. In muscles that are mildly affected by med, gastrocnemius and diaphragm, AChR levels rose only slightly, to about 3 fmol/mg. Thus, med-induced muscle inactivity prevents and reverses the normal postnatal decline in extrajunctional AChR levels.

We have also determined the effect of denervation on AChR levels in muscles from normal and med mice. Maximum levels were observed by 6d after denervation in both normal and med rectus. AChR levels in 15-20d old med mice that had been denervated for 6-11d rose from 11.9 to 29.8 fmol/mg in rectus and from 17.7 to 21.0 fmol/mg in biceps. In normal muscles under the same conditions, AChR levels rose to 35-40 fmol/mg. It is possible that the degree to which AChR levels rise after denervation is inversely related to the fraction of muscle fibers previously inactivated by the med disorder. We are currently testing this hypothesis.

These data do not exclude a role for activity-independent factors in the regulation of AChR levels in neonatal muscles, but they clearly show that nerve-induced muscle activity is important in the postnatal loss of extrajunctional AChRs.

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419.12 QUANTITATIVE STUDY ON SYNAPTIC AND RECEPTOR REORGANIZATION IN THE FELINE THALAMUS FOLLOWING LESIONS IN THE SUBSTANTIA NIGRA. K. Kultas-Ilinsky, B. Hughes*, C. Cuthbert*, S. Tewfik*, J. Fogarty* and I. Ilinsky. Department of Anatomy, University of Iowa College of Medicine, Iowa City, IA 52242.

It is well established that in the cat thalamus GABAergic afferents of nigral origin terminate in the ventral medial (VM) and ventral anterior (VA) nuclei. In this study an attempt was made to determine a sequence of synaptic and receptor changes in these thalamic nuclei induced by lesions in the substantia nigra (SN). In one group of adult cats, kainic acid injections were placed unilaterally in the SN and synaptic reorganization in the VM and VA was studied by electron microscopy. Quantitative light-microscopic receptor-binding autoradiography with ^{3}H-flunitrazepam (^{3}HF) as a ligand was performed on the same region of the thalamus in another group of similarly operated animals. Analysis of ^{3}HF binding parameters at different postlesion survival times demonstrated a significant increase in the number of binding sites (B max) in the VM and VA. The increase in Bmax values was detected in a week after lesion and persisted at the longest postlesion time analyzed (11 months). The EM results revealed the peak of degeneration in the thalamus on the 7th day after nigral lesions, followed by marked increase in glial apposition on the membrane of primary and secondary dendrites of thalamocortical neurons. The density of medium to large axon terminals with symmetrical contacts and pleomorphic vesicles decreased two-fold on these dendrites in the VA and about seven-fold in the VM. In 3 months after lesion, small size boutons with unusual features were noted to contact primary and secondary dendrites. The number of these boutons increased at longer survival times. These findings suggest sprouting of new terminals to the sites vacated by nigral afferents and provide first evidence on synaptic remodeling in the motor thalamus which is accompanied by changes in the number of benzodiazepine receptor sites. Although the source of the sprouting terminals remains unidentified, there are some indications that axons of GABAergic local circuit neurons may be responsible for this phenomenon.

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419.13 FATIGUE RESISTANCE AND OXIDATIVE CAPACITY OF THE DIAPHRAGM DURING POSTNATAL DEVELOPMENT. G.C. Sieck and C.E. Blanco*. Dept. of Anatomy and Brain Research Inst., UCLA Sch. of Med., Los Angeles, California 90024

The purpose of this study was to examine the postnatal changes in diaphragm (DIA) fatigability and oxidative capacity. Diaphragms were excised from animals at different ages between birth and 42 days of age. Strips of muscle were cut from the mid-costal region and suspended in a temperature controlled bath containing Kreb's solution. Isometric contractions were evoked by stimulation of the phrenic nerve, and fatigue resistance determined by repetitive stimulation at 40 PPS in 330 msec trains with 1 train presented each sec. A fatigue index (FI) was calculated as the ratio of initial force to the force generated after 2 min stimulation. Fatigue resistance of the DIA remained high through the first weeks (FI = 0.60 ± 0.10 in the neonate vs 0.58 ± 0.08 at 3 weeks). By 6 weeks of age, fatigue resistance decreased, approximating that of the adult muscle (FI = 0.40 ± 0.10 at 6 weeks vs 0.30 ± 0.07 in the adult). Diaphragm oxidative capacity was determined by measuring the activity of succinate dehydrogenase (SDH) both biochemically (Whole muscle estimate) and histochemically (single fiber estimate). Fibers were classified as type I or type II based on myofibrillar ATPase activity. During development there was a progressive increase in the proportion of type I fibers (from 10% in the neonate to 40% in the adult). The SDH activity of the neonatal DIA was the lowest of any age. The distribution of SDH activity among fibers of the neonatal DIA was very uniform (coefficient of variation, CV was 10% for type I fibers and 18% for type II fibers). By 3 weeks of age, SDH activity of the DIA increased, reaching its highest level. The CV of SDH activity among type I fibers remained low at 10%, whereas the CV among type II fibers increased to 34%. Between 3 and 6 weeks of age, SDH activity of the DIA decreased, but still remained higher than in the adult muscle. During the same period, the CV of SDH activity among both type I and II fibers increased, approximating that seen in the adult muscle (type I: CV = 18% at 6 weeks vs 22% in the adult; type II: CV = 44% at 6 weeks vs 53% in the adult). This increase in enzymatic variability during postnatal development most likely reflects the disappearance of polyneuronal innervation of fibers and the differentiation of different motor unit types. The time course for the changes in DIA fatigue resistance and oxidative capacity were not parallel, indicating that during postnatal development oxidative capacity and fatigue resistance are not tightly linked.

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419.14 DEVELOPMENTAL TRANSITION OF LOCOMOTOR PROGRAMS IN *APLYSIA CALIFORNICA*. M. Stopfer, G. Schuerman*, and T.J. Carew. Dept. Psychology, Yale University. New Haven, CT 06520.

It has recently been shown that a specific form of nonassociative learning, sensitization, emerges in the escape locomotion system of *Aplysia* quite late in juvenile development (Stopfer and Carew, 1987). To further examine the development of learning in this response system, it is important to understand the development of the behavior itself. Thus in the present study we have examined the development of locomotion throughout juvenile ontogeny.

Adult *Aplysia* locomote by means of a series of discrete retrograde pedal waves (steps). In contrast, young juveniles locomote by means of ciliary gliding (Kriegstein, 1974). We examined the transition in these locomotor programs throughout development, in the juvenile stages 9, 10, 11, Early 12 (E12) and Late 12 (L12). In the first phase of the experiment, animals' spontaneous locomotion was observed in glass tubes through a dissecting microscope for 3 min. Locomotion was scored (at 10 sec intervals), according to a 5 point scale: 0 = no locomotion; 1 = pure glide; 2 = modified glide (probing movements of anterior foot); 3 = rudimentary pedal wave (rhythmic contraction of the anterior foot, posterior gliding); 4 = pure pedal wave. Following the initial observation period, a noxious chemical stimulus was delivered to the animals' tails, which usually led to vigorous escape locomotion. Another 3 min observation period then began. Scores for each animal were averaged to yield a single locomotor index score for each observation period. The mean spontaneous locomotor index for stage 9 (N=4) was 1.35, indicating a predominance of pure ciliary gliding. Mean index scores for stages 10 (N=18), 11 (N=5), E12 (N=6) and L12 (N=7) were 1.59, 1.98, 2.38, and 3.00, respectively, revealing a significant developmental trend ($p<.01$) toward the adult form of locomotion. Thus, between stages 9 - 12, spontaneous locomotion showed a clear and gradual transition from juvenile to adult locomotor programs.

Escape locomotion differed from spontaneous locomotion in two ways: 1) it was much more rapid; and 2) at each stage it appeared to take on the form of locomotion typical of a later stage (e.g. young animals that only exhibited gliding during spontaneous locomotion could exhibit clear stepping in response to the noxious stimulus). These observations suggest that juvenile *Aplysia* have the neural circuitry required for mature (cyclical) locomotion long before it is spontaneously used.

In the adult, locomotion is mediated by a central program which can be triggered by serotonin and modulated by neuropeptides (Henning et al, 1979; Mackey and Carew, 1983). Since there is such a dramatic difference between juvenile and adult locomotion, it will be of interest to examine the juvenile locomotor circuit as it takes on these adult properties.

419.15 **PLASTICITY IN THE DENDRITES OF TRIGEMINAL MOTONEURONS OF Rana pipiens,** *B. M. Rosenthal and K. E. Alley, Dept. Oral Biology, The Ohio State University College of Dentistry, Col., OH 43210*

Dendritic reorganization of motoneurons has been studied in the invertebrate hawkmoth as the motoneurons are respecified to innervate new myofibers during metamorphosis (Levine and Truman, '85). Similar dendritic changes have not been studied in vertebrates which undergo metamorphosis but might be significant since these changes could underly alterations in behavior, such as the transition from filter feeding to predation in frogs. Metamorphic reorganization of the jaw in **Rana pipiens** is characterized by turnover of the jaw muscles; embryonic myofibers degenerate and secondary myofibers are born. During this transition, motoneurons are respecified to innervate new target cells, the adult myofibers. In this report we describe the changes in dendritic morphology by the trigeminal motoneurons that are respecified during myofiber turnover. Changes in feeding behavior from tadpole to adult are not explained by addition or subtraction of motoneurons, since motoneurons are not added to the trigeminal motor nucleus by neurogenesis or migration, nor are motoneurons deleted from the nucleus by cell death (Alley and Barnes, '83). It would be of interest to determine if the dendrites of trigeminal motoneurons are reorganized as are the abdominal motoneurons of hawkmoths. The branch of the mandibular portion of the trigeminal nerve supplying the posterior levator mandibularis muscle was transected and labeled with HRP. In adult frogs the dendrites of trigeminal motoneurons ramify in two spatially discrete zones including the ventrolateral and the dorsomedial medulla (Matesz and Székely, '78). In contrast, we found that the dendrites of the motoneurons from premetamorphic tadpoles are oriented almost exclusively in the ventrolateral medulla. In addition, we have observed developmental changes in the somatodendritic geometry in premetamorphic tadpoles including increases from stage V to stage XII in 1) number of primary dendrites, 2) diameter of primary dendrites, 3) length of dendrites, 4) size of the trigeminal nucleus and 5) somal volume. As these motoneurons are recycled during the transition from larva to adult, their dendrites have the potential for a dramatic increase in synaptic connectivity as a mechanism to establish the new feeding behavior present in the adult.

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419.16 **THE TIMING OF SWIMMING IS CONTINGENT ON METAMORPHOSIS FOLLOWING RECOVERY FROM SPINAL TRANSECTION IN THE SEA LAMPREY.** L. Margolin* and J. Ayers. (*Spon: R. Schatz*). Dept. of Biology and Marine Science Center, Northeastern University, East Point, Nahant, MA 01908.

Temperature has been shown to have important effects on the quality of recovery from spinal transection in larval sea lamprey (*Soc. Neurosci. Abstr. 12: 1574*). In the present experiments we have extended these observations to metamorphosing sea lampreys, to establish a contingency on developmental processes.

Full spinal cord transections were performed on experimental animals at 25% of total body length. Specimens were then placed in individual aquaria where they were allowed to recover at either 13° or 21°C until criterion for complete recovery of swimming and other behaviors (*Brain Res. 279: 238*) was attained. Episodes of recovered free-swimming were filmed, digitized, and analyzed by a computer algorithm (*Science 221: 1312*) which computed the amplitude and timing parameters of swimming behavior as a percent of that observed in normal specimens of the same size.

Temperature effects on timing appear to be more profound on specimens that transform as they recover than on specimens transected after metamorphosis. Specifically, the effect of temperature on pre-metamorphic specimens parallels the changes observed in period, propagation time, and phase lag that are observed in ammocoetes (*Soc. Neurosci. Abstr. 12: 1574*). The specimens that recovered at the colder temperature took longer but recovered more normal behavior. In contrast, post-metamorphic specimens exhibit changes in timing that are opposite from that seen in larvae. There was no difference in amplitude for both pre- and post-metamorphic specimens at the two temperatures (as in ammocoetes)

The above results indicate that metamorphosis has a greater effect on the timing of recovered swimming undulations, than does temperature. This contrasts with the greater effect of temperature on the chronology of recovery and the negligible influence of development stage *(Wilbur, et al., this volume). Supported by NSF Grant BNS-8406880.*

419.17 LAMINAR ORIGINS AND SPINAL TERMINATIONS OF IPSILATERAL CORTICOSPINAL PROJECTIONS IN THE NORMAL RAT. D.R. Humphrey & W.F. Nation* (SPON: M. Waitzman), Dept. Physiol., Emory Univ., Atlanta, GA 30322

A substantial ipsilateral corticospinal (CS) projection is known to issue from the surviving motor cortex of adult rats subjected neonatally to ablation of the opposite motor cortex (Hicks and D'Amato, Exp. Neurol., 29:416, 1970). It has also been shown with modern axonal tracing methods that a small ipsilateral CS projection exists in the normal animal (Sievert and Neafsey, Neurosci. Abstr., 11:1286, 1985). In this study, we obtained further information about the cortical origins, course and spinal terminations of the ipsilateral CS projection in the normal adult rat. To trace the pathway anterogradely, ten, 0.1 uL injections of 2% WGA-HRP were made within the forelimb motor areas of one hemisphere. The animals (N=6) were sacrificed 48 hr later, and frozen sections of the brain and spinal cord were processed with TMB for visualization of labeled CS axon terminals. To study the cortical origins of the projection, small gelfoam pledgelets saturated with 2% WGA-HRP were inserted into one cut CS tract at the C6-C7 level of the cord, after transection of the opposite tract at C2 (N=3); thus, only one dorsal column CS tract existed for retrograde transport of the label. As a control for possible diffusion of the tracer to, and pickup by, uncut CS fibers, injections of Fluorogold (2%) were also made unilaterally into the cervical gray matter, opposite to the C2, CS tractotomy; Fluorogold is presumably not picked up by intact fibers of passage (N=3 animals). The animals were sacrificed two days (WGA-HRP animals) or seven days (Fluorogold animals) after injection of the tracer, and their cerebral cortices were examined for the presence of retrogradely labeled neurons.

Our major results were as follows. (1) Ipsilaterally coursing CS fibers were found in ventral and dorsal columns, chiefly in the latter. (2) Unlike the crossed projection, which terminates principally in the dorsal horn (laminae IV-VI), ipsilateral CS fibers teminated chiefly in the spinal intermediate zone (lamina VII). (3) Ipsilaterally projecting CS neurons were found in all of the major zones which give rise to crossed projections (both forelimb zones and somatosensory area II). (4) Ipsilaterally projecting cells resided in the ventral one-third of cortical lamina V, whereas contralaterally projecting cells are distributed throughout lamina V. (5) The ratio of contralaterally to ipsilaterally projecting cells is on the order of 20:1.

Thus, a small but significant ipsilateral CS projection issues from a subset of cells in lamina V, and terminates in the intermediate zone of the spinal cord. The enhancement of this projection through axonal sprouting or other processes may underlie in part the much larger and stronger ipsilateral projection seen in the neonatally lesioned animal. (Supported by NIH Grant NS 20146).

419.18 CHARACTERISTICS OF CORTICOSPINALLY MEDIATED, IPSILATERALLY EVOKED MOTOR RESPONSES IN THE NORMAL ADULT RAT. W.F. Nation* and D.R. Humphrey (SPON: D. Eaton). Dept. Physiol., Emory Univ., Atlanta, GA 30322.

Previous studies have established that intracortical microstimulation (ICMS) within the surviving motor cortex will evoke bilateral motor responses, in an adult rat subjected neonatally to ablation of one motor cortex. It has also been shown (Humphrey and Nation, this volume) that an ipsilateral corticospinal (CS) projection exists in normal adult rats, which may be a substrate for the enhanced projection seen in neonatally lesioned animals. Some controversy exists, however, concerning the functional strength of this pathway in the normal animal: some workers have reported the existence of ipsilaterally evoked responses of neck and proximal limb muscles, whereas others have failed to detect such responses when using ICMS, even with currents up to 100 uA intensity. It has recently been shown, however, that weak motor responses that are not evoked by ICMS methods following pyramidotomy, may be 'uncovered' with the use of stimulating electrodes with larger tip exposures (Mitz, A.R. and Humphrey, D.R., Neurosci. Lett, 64: 59, 1986). This occurs, presumably, because the cells which give rise to these responses are sparsely distributed within the cortical area stimulated, and cannot be activated in sufficient numbers with more localized currents, unless they are raised to injurious densities at the site of application. We have therefore used intracortical stimulating electrodes with exposed tip surfaces some 5-7 times larger than those used with ICMS methods (400-500 um^2 versus 70-100 um^2) to map the motor cortex of the normal adult rat, in search of ipsilaterally evoked motor responses. The experiments were performed on 6 animals, under light (7-10 mg/Kg) ketamine sedation.

Our major results were as follows. (1) Ipsilateral motor responses were evoked with current pulse trains of 30-100 uA intensity (300 pps, 0.2 msec pw), even though such responses were not seen with equivalent currents (and 5 times the current density) using ICMS. (2) The ipsilaterally evoked responses were less discrete, involving more muscles and joint movements, than those seen contralaterally with ICMS. (3) The ipsilateral responses occurred principally in axial and proximal limb muscles, with no evoked digit responses, with the latter being confined to the contralateral side. (4) All evoked motor responses were abolished by medullary pyramidotomy (N=2) or corticospinal tractotomy at the C2 level (N=2). We conclude, therefore, that a functional ipsilateral corticospinal projection exists in the normal rat, and suggest that its enhancement through axon collateral sprouting or other mechanisms may contribute substantially to formation of the more powerful ipsilateral system that exists in the neonatally lesioned animal. (Supported by NIH Grant NS 20146).

419.19 DISCHARGE PROPERTIES OF FAST AND SLOW MOTOR UNITS DURING VOLUNTARY AND REFLEX CONTRACTION OF WRIST EXTENSOR MUSCLE IN MAN.
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Experiments were performed on 20 human subjects, all of whom gave informed consent to experimental procedure.

Slow and fast motor units (MU) of the extensor carpi radialis longus muscle were identified, depending on the contraction time of the twitch extracted from the global muscle tension by the spike-triggered averaging method. Single muscle fibre activity was recorded using a tungsten microelectrode (.5 - 4 MΩ)during isometric muscle contraction which was either voluntarily performed or reflexely induced by tendon vibrations (50-80 Hz). MU discharge was analysed using an instantaneous frequency meter.

The contraction times of the MU twitches ranged from 15 to 70 ms, the majority (53%) lying between 25 and 35 ms. The twitch force was about 5 mN to 20 mN.

Each MU which was triggered by an intentional muscle force increase (0-10 N) performed at low velocity (.1 $N{\cdot}s^{-1}$) began to fire at a precise force level. As from 1 N, the majority of the MUs had short twitch contraction times (15-35 ms), whereas the MUs discharging at lower forces were characterized by longer twitch contraction times. MU maximal discharge frequencies ranged between 15 and 30 Hz and were characterized by a noticeable variability of spike time intervals.

Instantaneous discharge frequency analysis showed that slow MUs reached their maximal discharge almost immediately, whereas fast MU activity increased more progressively. Fast MUs thus appeared to be able to modulate muscle force within a small range, as observed when the subject performed sinusoidal force variations with varying amplitudes. Force increases performed more and more rapidly did not modify the chronology of MUs recruitment, but made them fire at a lower force level without modifying their maximal discharge frequency. During muscle relaxation, MU activity decreased with a longer time constant thant that which characterized their discharge onset.

Tendon vibrations applied to a sligthly contracted muscle (so that supraspinal and peripheral motoneuronal inputs could be combined) were able to drive every MU discharge without any latency. When applied to relaxed muscle, vibrations generally induced MU discharge after several seconds. During both voluntarily performed and tendon vibration induced muscle force increases at the same constant velocity, the MU discharge onset occurred strictly at the same force level.

This exhaustive analysis of the motoneuronal command of the wrist extensor muscle should provide a basis for further studies on the supraspinal coding of isometric and isotonic muscle contraction and joint movements and their regulatory mechanisms in subjects subjected to various behavioural situations.

419.20 STAGES OF FUNCTIONAL RECOVERY IN THE ADULT CAT FOLLOWING THORACIC HEMISECTION.
Maureen E. McBride,* Joseph Olivier* and Michael E. Goldberger. Dept. of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA. 19129.

Previous studies have documented relatively few persistent motor deficits after chronic hemisection in cats, implying that significant post-lesion recovery occurs. Examination of the immediate sequelae of the lesion may elucidate the mechanisms responsible for this recovery. Daily examination of motor function during the acute period following T13 hemisection sparing the dorsal columns, reveals a sequence of changes that depicts stages of recovery. Behaviors studied were: 1) conditioned overground locomotion on a number of runways of increasing difficulty. "Crude" locomotion on a 30 cm wide runway; "precise" locomotion on a narrow 5cm runway, lattice board, ladder, and peg board; 2) reflex locomotion on a motor driven treadmill; 3) placing and hopping responses. Kinematic analysis and threshold measurements were used to evaluate these behaviors. Ipsilateral to the lesion tactile placing is abolished permanently. Proprioceptive placing and monopedal hopping are absent until the 3rd postoperative day when these responses begin to appear; initially the thresholds are elevated and responses hypermetric. Crude bipedal locomotion on the 30cm runway and treadmill-elicited locomotion were observed by the 2nd postoperative day - although with abnormal placement of the ipsilateral hindlimb on the dorsum of the toes. By the end of the first week monopedal reflex thresholds and responses progressively decrease toward more normal values, and a plantigrade stance begins to recover, as does locomotion on the 5cm runway, which requires accurate foot placement. The step cycle pattern obtained from measurements of joint angle excursions for the hip, knee, and ankle is altered during the recovery period. These kinematic changes are associated with the following stages of motor recovery: 1. recovery of crude locomotion, 2. recovery of lowered thresholds and greater control in the execution of postural reflexes, 3. recovery of locomotion demanding precise movement.

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419.21 THE EFFECT OF DENERVATION ON SKELETAL MUSCLE MYOSIN ISOFORMS IN FAST- AND SLOW-TWITCH MUSCLES OF C57BL/6J MICE.
G. Desypris, H.L. Davis and D.J. Parry. Department of Physiology, University of Ottawa, Ottawa, Canada; and Department of Anatomy and School of Physical and Occupational Therapy, McGill University, Montreal, Canada.

Denervation of fast-twitch skeletal muscle results in slowing that is manifest as a reduction in the maximal rate of rise of tension as measured under both isometric and isotonic conditions. In the early phase of denervation the slowing has been attributed to an alteration in the calcium sequestering capability of the sarcoplasmic reticulum. After longer periods of denervation, the presence of fibers containing slow isoforms of myosin contributes further to the slowing. This study was undertaken to compare the effect of denervation on the isoforms of myosin in the soleus (SOL), a slow-twitch muscle, and the extensor digitorum longus (EDL), a fast-twitch muscle of the C57BL/6J mouse after short (1 month) and longer (3 months) periods of denervation.

The right hindlimbs of 4-5 month old anesthetised C57BL/6J mice were denervated by surgical removal of a segment of the sciatic nerve in the upper thigh region. Myosin isoforms were detected and visualised immunohistochemically. Monoclonal antibodies directed against the heavy chain of IIA and IIB forms of fast myosin as well as slow myosin (generous gift of Dr. S. Sciaffino, Padua, Italy) were applied to frozen cross-sections of contralateral control (CON) and 1 and 3 month denervated (DN) SOL and EDL muscles. Serial sections were stained with hematoxylin and eosin for fiber counting. Isometric twitch temporal characteristics of time to peak tension (TTP) as well as half-relaxation time (1/2 RT) were measured on one month denervated (n=5) as well as age-matched untreated (n=7) EDL. At one month post-DN both TTP and 1/2 RT of DN EDL were significantly prolonged compared to untreated EDL. (TTP: 12.2 ± 1.0 vs 7.1 ± 0.4 msec, and 1/2 RT: 14.9 ± 2.5 vs 7.2 ± 0.5 msec respectively. All values represent mean ± standard deviation). No increase in the proportion of fibers containing slow myosin was noted, however the presence of hybrid fibers containing both IIB and IIA myosin was seen in DN EDL. By 3 months after DN a striking loss of IIB myosin was noted in the EDL. The proportion of fibers containing IIA myosin was increased in DN EDL, and in a few instances a slight increase in slow myosin was also seen. The increase in slow myosin was more dramatic in DN SOL. No difference in fiber number was noted between DN and contralateral CON muscles. It is postulated that in fibers of EDL muscles which contain type IIB myosin, a switch to IIA myosin synthesis is triggered by denervation. In DN SOL, fibers that previously synthesized IIA myosin begin to synthesize slow myosin. The fact that no significant increase in slow myosin was detected in EDL muscles by one month post-DN supports the idea that the early phase of slowing is due to factors unrelated to alterations in myosin isozyme composition.

Supported by MRC

419.22 ULTRASTRUCTURE OF SKELETAL MUSCLE IN TURNER´S SYNDROME.
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Patients with Turner´s syndrome usually have short stature, suffer from lack of muscle strenght and examination may reveal reduction in muscle bulk. In order to investigate the influence of this chromosomic abnormality on the ultrastructure of skeletal muscle, needle biopsies were taken from quadriceps femoris muscle, in two patients, 19 and 25 years old.

In both patients muscle was affected; the alterations found were: 1. Atrophy, which varies from slight to severe. There is a loss of myofibrils with widening of intermyofibrillar spaces that appear filled with glycogen particles. In the most severe cases exists total disorganization of muscle architecture. 2. Presence of cytoplasmic bodies located in the subsarcolemmal space. 3. Some mitochondria show tubular christae and a low electron density. 4. There is an increase of lysosomal activity with presence of multivesicular bodies and proliferation of lipofuscin granules. 5. Some muscle capillaries look occluded and present vacuoles in the cytoplasm. Endothelial wall is thinnned in many places. 6. Frequent presence of macrophages in the extracellular space.

Our results give an ultrastructural histopathological basis to the clinical alterations of skeletal muscle in Turner´s syndrome.

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419.23 **STUDIES OF BEAM-WALKING IN RATS: IMPLICATIONS FOR AMPHETAMINE FACILITATED MOTOR RECOVERY AFTER BRAIN INJURY.** L.B. Goldstein*, G.A. Miller*, N.M. Cress*, and J.N. Davis. (Spon. I.T. Diamond) V.A. Medical Center and Depts of Medicine (Neurology) and Pharmacology, Duke Univ., Durham, NC 27705

Amphetamine administration enhances the rate of recovery of beam-walking in rats after cortex lesions. We have been studying the suitability of beam-walking in rats as a model for predicting the effect of drugs on recovery of function after stroke. We recently demonstrated that the number, but not the timing, of trials on the beam is critical to amphetamine-facilitated recovery. "Prodding" the animals alone promoted recovery but did not enhance the amphetamine effect.

In the present experiments, the effects of trial interval, prodding and pre-training on beam walking scores were studied. All ratings were performed by blinded observers using a seven point scale with excellent inter-observer agreement (Kappa > .93).

Sham operated control rats showed a significant fall-off in performance when six trials were given sequentially over 12 minutes. This decrement could be eliminated by "prodding" and reduced by pre-training or longer trial intervals. Confirming our previous work, lesioned animals tested sequentially showed an increased rate of recovery when subjected to "prodding" alone. The improvement persisted 24 hours after the last sequential trial.

These results indicate that: (1) sham operated controls normally show a decrement in beam-walking performance when subjected to frequently repeated trials, (2) the fall-off in performance could be eliminated by "prodding" control animals and, (3) "prodding" alone improved recovery in lesioned animals. These data in conjunction with our previous experiments show that the way in which beam-walking is performed can influence the effect of pharmacological agents on recovery of function.

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419PO RECOVERY RATE OF NERVE CONDUCTION IN ACUTE REVERSIBLE CONDUCTION BLOCK OF EXPERIMENTAL NEUROPATHY. C-Z. Hong and J. Yu. Department of Physical Medicine and Rehabilitation, University of California, Irvine Medical Center, Orange, CA 92668.

The recovery of experimental compression neuropathy with prolonged conduction block of the caudal nerve was studied electrophysiologically in 24 adult Sprague-Dawley albino rats in three equally divided groups. The pressure applied in group A, B, and C was approximately 20 Kg/cm^2 for 5 minutes, 10 Kg/cm^2 for 20 minutes and 10 Kg/cm^2 for 5 minutes respectively. The nerve conduction measurement on the caudal nerve was taken before and after compression. The nerve was stimulated both proximally and distally to the site of compression, and the compound muscle action potential (CMAP) was recorded with a surface electrode from the segmental muscles of the tail. The motor conduction velocity (NCV) of the compression segment, and the ratio of the proximal/distal CMAP-amplitude (p/d AMP) were calculated to evaluate the degree of demyelination and conduction block of the compressed nerve respectively. The period of initial complete block was significantly longer in group A (9.5±4.6 days) than in group B or C, but no significant difference occurred between groups B and C (both 2.4±0.5 days). Once the conduction block began to recover, the recovery rate of conduction block (recovery rate of P/d AMP) was not significantly different among the three groups. The recovery rate of demyelinative lesion (recovery rate of NCV) was not significantly different among the three groups either. Complete recovery of both NCV and P/d AMP was noted after 37.5±5.9 days in group A, 31.3±4.2 days in group B and 29.4±3.0 days in group C. This study provides an animal model for investigating therapeutic effects of various interventions on neuropathy.

419PO RECOVERY OF COORDINATED STEPPING IN RATS WITH SPINAL CORD TRANSECTIONS DONE AT DIFFERENT POSTNATAL AGES. J.W. Commissiong and G. Toffano. Dept. of Physiol., McGill Univ., Montreal, Canada H3G 1Y6 and Fidia Res. Lab., 35031 Abano Terme, Italy.

Mammalian spinal locomotor generators (SLGs) can control some aspects of reflex, locomotor functions of a limb, independently of the higher centres. The experiments were done to test the plasticity of SLGs in rat during development. The cord was transected at postnatal ages: PN 0 (4 hrs), 7, 14, 21 and 28 (N=5). At 18 weeks after birth, two rats from each of the 5 groups plus two unoperated controls, were put in six cages labelled A to F, and scored on a scale of 0 (worst) to 10 (best) for four-limb coordinated walking ability by 37 first year medical students. Due care was taken to ensure reliable scoring. The results indicate the number of students that assigned a given score to each experimental group. Animals transected at PN 14, 21 and 28

DISTRIBUTION OF SCORES (0-10) FOR FOUR POINT MOTOR ABILITY IN RATS CHRONICALLY SPINALIZED AT DIFFERENT POSTNATAL AGES

Transection Day	SCORE 10	9	8	7	6	5	4	3	2	1	0	N
C	[37]	0	0	0	0	0	0	0	0	0	0	37
0	0	1	1	5	[12]	5	5	4	3	1	0	37
7	1	3	[20]	3	2	2	2	2	1	1	0	37
14	0	0	0	2	4	4	[11]	6	8	1	1	37
21	0	0	0	1	4	4	5	5	[13]	5	0	37
28	0	0	0	0	0	0	1	0	2	0	[34]	37

The spinal cord was totally transected at 0 (4 hrs), 7, 14, 21 and 28 days after birth, at the middle thoracic level. The evaluation of motor ability was done at 18 weeks after birth.

remained overtly paraplegic, (modal scores: 4, 2 & 0 respectively); while the PN 0 and PN 7 groups showed substantial recovery (modal scores: 6 & 8 respectively). The rank order of the scoring, with the range indicated in parenthesis was: unoperated controls (10-10) > PN 7 (1-10) > PN 0 (2-9) > PN 21 (1-7) > PN 28 (0-4). Recovery was optimal in the PN 7 group. The critical time beyond which no, or only minimal recovery occurs, is PN 14. There was an excellent correlation between the degree of recovery of reflex stepping ability, and the concentration of dopamine in the caudal, lumbar cord. Preliminary results of the testing of the excitability of the motorneuron pools in the lower lumbar and upper sacral regions of the cord, indicate that there are no marked differences in motoneuronal excitability among the different experimental groups.

420.1 BIOCHEMICAL AND MORPHOLOGICAL PROPERTIES OF MICROGLIA IN CULTURES OF RAT CEREBRAL CORTICAL CELLS. W.E. Thomas, J.M. Pratt*, H.J. Wynder* and F.L. Jordan. Departments of Physiology and Anatomy, Meharry Medical College, Nashville, TN 37208.

While microglial cells are considered intrinsic macrophages and thought to be involved in the immune response in brain tissue, many aspects of their cellular function are unclear. These cells have not been highly investigated in CNS cultures containing mixed population of cells. Microglia were investigated here in tissue cultures of dissociated cerebral cortex from embryonic rat. Three different forms of microglial cells were identified in relatively mature cultures on the basis of morphological criteria, esterase histochemistry, and immunohistochemical staining for Fc receptors and macrophage-associated surface components. Two of these forms corresponded to the amoeboid and ramified microglia describe in vivo; the third form appeared to be unique and was distinguished by its morphological resemblance to macrophages. This latter form was characterized by the presence of numerous filopodial processes.

The amoeboid microglia stained intensely for non-specific esterase activity, while Fc receptors and macrophage-associated antigens did not appear to be present on this form. The ramified cell possessed little or no esterase activity, but stained well for Fc receptors and appeared to express some macrophage antigen. The third, unique morphological form possessed moderate non-specific esterase activity and stained comparably for Fc receptors and macrophage-specific components. Using latex beads (1-5 μm diameter) to assess phagocytic activity, only the amoeboid and filopodia-bearing forms exhibited this capability. The filopodia-bearing form was the predominate type of microglia observed in the cultures; however, the exact nature of its relationship to the amoeboid and ramified cells is uncertain. It can not be distinguished at present whether this form corresponds to the "reactive" microglia described in vivo which is thought to be derived from the ramified cell or represents an intermediate between the amoeboid and ramified cells. Moreover, the identification of these cell types supports the potential use of this culture system to investigate functional aspects of microglia and their interaction with other brain cells.

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420.2 DETERMINATION OF THE SOMATOSTATIN-CONTAINING NEURONAL POPULATION IN PRIMARY CULTURES OF CEREBRAL CORTEX. F.L. Jordan and W.E. Thomas. Department of Physiology, Meharry Medical College, Nashville, TN 37208.

Somatostatin was initially identified in the hypothalamus as an inhibitory factor for the release of growth hormone, and subsequently shown to arrest the release of biologically active substances in several other tissues. Extra-hypothalmic regions of the brain also contain somatostatin. Based on several lines of evidence somatostatin has been suggested to function as a transmitter of neurons intrinsic to the cerebral cortex; however, the synaptic physiology of somatostatinergic neurotransmission has not been characterized. As an initial step in the characterization of the physiological role of neuronal somatostatin in cortex, the antigenic expression of this peptide was examined in primary cultures of rat cerebral cortical cells. In this preparation, the exact number of peptide-containing neurons could be determined and a complete morphological assessment of this neurochemical cell group performed.

Somatostatin-like immunoreactivity was detected using an indirect immunofluorescent staining technique. Specific immunoreactivity appeared to be localized only in neurons. Staining activity began to appear in some neurons in the cultures during the second week in vitro and increased in intensity over the third week. The proportion of this somatostatin-containing neuronal subpopulation was determined by direct cell counting. A percentage of 1.25 of the total neurons contained somatostatin based on multiple counts from six different cell platings. Somatostatin cells corresponding to the four major morphologies of pyramidal, stellate, bipolar and bitufted were detected; however, the majority of stained neurons exhibited bipolar (61%) or stellate (35%) morphology. While these bipolar and stellate cells varied slightly in their detailed morphology, they were consistently small in size. All were less than 20 μm in somal diameter, and most were less than 15 μm. These findings define the number and nature of putative somatostatinergic neurons in this cortical culture system. The morphological features should facilitate the identification of these cells for physiological investigation in viable cultures.

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420.3 A MORPHOLOGICAL CHARACTERIZATION OF THE GABA-ERGIC NEURONAL POPULATION IN TISSUE CULTURES OF CEREBRAL CORTICAL CELLS FROM RAT. B.W. Hughes*, F.L. Jordan and W.E. Thomas (SPON: J.G. Townsel). Division of Biomedical Sciences and Department of Physiology, Meharry Medical College, Nashville, TN 37208.

The role of gamma-aminobutyric acid (GABA) as a transmitter of local circuit inhibitory neurons in cerebral cortex has been well established; however, the full morphological complement of GABA cells in this tissue has not been extensively investigated. In the present study, a primary tissue culture system of rat cerebral cortex has been employed as an amenable preparation in which to completely define morphological subtypes of GABA neurons. The GABA neurons were labelled in these cultures by GABA-uptake autoradiography. In this procedure viable cortical cultures were incubated with ^{3}H-GABA, fixed and then processed for autoradiographic visualization. Resulting autoradiograms were mounted on slides, viewed with light microscopy, and labelled GABA cells were counted and classified.

In a preliminary series of experiments, the kinetics of GABA uptake by the cultures were investigated. Using varying concentrations of ^{3}H-GABA, transport of the transmitter displayed an approximate K_T of 0.5 μm. At a concentration of 0.1 μm, uptake of label appeared to saturate within 10 min. Therefore, in subsequent efforts to maximally label the total GABA neurons population for autoradiography, cultures were incubated with 0.5-1.0 μm ^{3}H-GABA for at least 10 min.

The GABA-ergic neurons were a subpopulation of the total neurons and were initially classified using four major morphological groups - pyramidal, stellate, bipolar and bitufted cells. While GABA cells of all four morphologies were observed, the majority of labelled cells were stellate or bipolar (>90%). Stellate cells (51%) slightly exceeded the proportion of bipolar cells (43%). Subtypes of labelled cells were observed in each of these two major morphological groups. Seven different subtypes of stellate cells were distinguished on the basis of soma shape, corresponding to star-shaped, cuboidal, small round, large round, pear-shaped, oblong, and trianglular. These seven stellate subtypes were rather evenly represented with the star- and pear-shaped and large round being relatively predominate. Bipolar cell subtypes were characterized on the basis of soma size as small (<10 μm), medium (10-20 μm), and large (>20 μm). The small (55%) and medium cells (36%) dominated this group. These studies established the complete morphological nature of GABA-ergic neurons in the cortical cultures and may provide the basis for future physiological investigations.

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420.4 A CHARACTERIZATION OF EPENDYMA FROM EMBRYONIC RAT CEREBRAL CORTEX IN VITRO FACILITATED BY A NOVEL STAINING ACTIVITY. H.J. Wynder*, G.K. Rieke, B.W. Hughes*, F. L. Jordan and W.E. Thomas (SPON: H. Rucker). Departments of Anatomy and Physiology, Meharry Medical College, Nashville, TN 37208.

The functional role of ependymal cells which form an epithelium held together by junctional complexes and line the ventricles of the brain has not been completely resolved. In an effort to develop a model system with which to investigate the cellular function of brain ependyma, the development and ultrastructure of these cells was examined in primary tissue cultures of cerebral cortex. The initial identification and characteriztion of ependymal cells in these cultures was greatly facilitated by a novel form of immunohistochemical staining. A commercial antiserum against vasoactive intestinal polypeptide (VIP) was found to selectively stain ependyma via indirect immunofluorescence. This staining was not specific for VIP and appeared to be attributable to a secondary antibody in the serum preparation; however, the staining provided a convenient means for visualizing these cells.

The ependyma arise during the second week in these cultures and continue to proliferate into the third week. They comprise a small component of the total cell population and, consistent with the ependyma in vivo, exhibit three major morphological forms -cuboidal, tanycyte and secretory cells. All three cell forms appeared to develop from a common precursor. When the ultrastructure of ependyma was examined with electron microscopy, cilia, microvilli, potential secretory structures and frequently a characteristic pore marked the apical cell surface. The cytoplasm contained profuse filamentous material and the lateral margins of the cells exhibited junctional specializations. These features closely resemble their cellular counterparts in vivo, and support the functional integrity of ependymal cells in this culture system.

Thus, ependymal cells have been characterized in vitro and these studies were greatly facilitated by selective immunohisto chemical staining which temporally preceded the expression of authentic VIP. The ependymal cell staining was most intense in the cilia and also generally intense in the nucleus. Staining was diffusely distributed over the remainder of the cell and revealed aspects of its morphology. Studies aimed at characterizing the ependyma-associated antigen(s) are in progress.

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420.5 PROTEIN SYNTHESIS IN EXPLANTED CHOROID PLEXUS WITH REFERENCE TO APOCRINE SECRETION D. Gudeman, E. Merisko*, M. Brightman° and C. Merril. (Spon. E. Gershon) *Dept. Anatomy Kansas Univ.Med.Sch., Kansas City KS 66103. °Lab.of Neurobiology, NINCDS, NIH and Lab of Biochemical Genetics, NIMH, Bethesda, MD 20892. The route proteins take into the cerebrospinal fluid (CSF) remains an open question. A working hypothesis is the CSF protein profile is produced by ultrafiltration of blood proteins coupled with the addition of proteins synthesized by the choroid plexus. Morphology of the choroid plexus (CP) suggests that secretion could occur by a mechanism of apocrine secretion. There is evidence that apically derived membrane vesicles (aposomes) that are produced during apocrine secretion will incorporate S^{35} labeled amino acids. It is the purpose of this work to use explanted choroid plexus from rat with in vitro labeling to study the apocrine secretory process. CP from 3 rats were incubated in 95% O_2, 5% CO_2 37°C, in 100ul of Hepes buffered simulated CSF. After 1 hour, 50 ul of media was removed and monitored microscopically to be cell-free. S^{35} methionine and S^{35} cysteine (120uCi) was added with 100ul of incubation media to both fractions. TCA precipitable counts were monitored over a 5-6 hour period. Measurable counts appeared at 3 hours. Protein profiles were determined through 2-dimensional SDS-PAGE followed by silver staining and fluorography. Equal amounts of TCA precipitable counts from the two fractions were loaded. The protein profiles were compared to that of rat CSF protein profile. The most heavily labeled spot in the tissue fraction migrated at the approximate isoelectric point(pI) of 6.3 and apparent molecular weight(M_r) 14kd. This spot appeared in trace amounts in the aposomal fraction although it silver stained heavily in the CSF fraction. This is the proper location for the transthyretin subunit, a previously known constituent of CSF. Furthermore, two heavily labeled spots migrating at approximately M_r 50 kd, pI 6.0 and M_r 43 kd, pI 5.8, appeared in both preparations. The fact the amount of synthesis of a major protein appeared specifically decreased in the aposomal fraction while other proteins were visualized in amounts comparable to those of epithelial cells, implies that the aposomes synthesize a protein profile that is a subset of the protein profile produced by the choroid plexus epithelial cells. This difference may be a manifestation of an intracellular partitioning event that is evident morphologically during apocrine secretion.

420.6 HETEROGENEITY OF POMC mRNA CONTENT IN PITUITARY ANTERIOR LOBE CORTICOTROPES AND INTERMEDIATE LOBE MELANOTROPES. J.M. Hatfield*, J.P. Adelman*, J. Douglass*, C.T. Bond*, and R.G. Allen. Dept. of Medicine/Biochemistry and Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences Univ., Sch. of Med., Portland, OR 97201

Recently, heterogeneity of proopiomelanocortin (POMC) mRNA content in pituitary anterior lobe corticotropes and intermediate lobe melanotropes has been demonstrated by in situ hybridization of tissue sections (Chronwall, et al.,Endocrinology,120:1201,1987). We have tested for heterogeneity of POMC mRNA content in acutely dispersed rat corticotropes and melanotropes. Trypsin dispersed cells were allowed to settle and adhere to poly-L-lysine coated coverslips prior to paraformaldehyde fixation and subsequent PBS rinses. Fixed cells were hybridized with ^{35}S-labeled POMC or 1B15 riboprobes. Studies of 1B15 mRNA cell content were undertaken to insure that the apparent cellular POMC heterogeneity is not inherent to in situ hybridization. 1B15 mRNA has been found at comparable levels in all tissues tested and is thought to be a "house keeping" gene expressed at similar levels in all cells (Milner and Sutcliffe,Nuc. Acids Res.,11:5497,1983). Such mRNAs would be expected to have a homogeneous distribution relative to mRNAs from hormonally regulated genes such as POMC. Thus, 1B15 was used to control for methodologic sources of grain density heterogeneity. Five % of the cells in the anterior pituitary lobe cell suspension and 95% of the cells in the intermediate lobe cell suspension were positive for POMC mRNA. Extensive heterogeneity of silver grain densities were seen over POMC mRNA containing cells from both lobes. The spacial separation of the cells on coverslips augments this apparent heterogeneity by preventing emission tracking from adjacent cells as occurs in tissue sections. In preliminary experiments, there appeared to be little, if any, heterogeneity of 1B15 mRNA content in cells from either pituitary lobe. Therefore, the corticotrope and melanotrope heterogeneity appears to be due to large differences in POMC mRNA content. Further study will be required to determine the significance of this finding. This in situ hybridization method will complement tissue section methodologies, required for morphological localization, by providing better quantitation of mRNA content of hormonally and developmentally regulated genes. This method should be equally useful for dissociated tissues and cultured cells. This work was supported by NIH grants HD18438 and NS19397 (to RGA).

420.7 CLONAL CELL LINES FROM NEUROBLASTOMA - SPINAL CORD CELL HYBRIDIZATION. N.R.Cashman,* S. Boulet*, and J. Antel* (Spon: R. Zatorre). Montreal Neurological Institute, Montreal, Quebec, Canada H3A 2B1

The basis for selective vulnerability of motor neurons in amyotrophic lateral sclerosis is unknown. Investigation of motor neuron cell biology has been hampered by poor yields and limited purity of primary cell preparations. We have isolated 39 cloned cell lines from a series of neuroblastoma-spinal cord cell fusions in an effort to develop proliferating cell lines which express some differentiated characteristics of motor neurons. Day 12-14 embryonic CD-1 mouse spinal cords (stripped of meninges and dorsal root ganglia) were dissociated with trypsin and DNAse; cells were cultured 1-2 hours to reduce fibroblast and astrocyte contamination. The fusion partner was the mouse neuroblastoma N18TG2, which lacks hypoxanthine phosphoribosyl transferase (HPRT) and is maintained in 6-thioguanine (6-TG) to eliminate spontaneous revertants. N18TG2 cells, cultured for 1 week without 6-TG, were trypsinized and replated, and overlaid with a 5-10 fold excess of spinal cord cells suspended in phytohemagglutinin-P. The attached cells were exposed to 50% polyethylene glycol (PEG) 1000 for 30 to 90 seconds (a 120-second exposure yielded no viable colonies) and washed repeatedly. Fusion products were mechanically dissociated and plated in hypoxanthine-aminopterin-thymidine (HAT) medium the next day. Neuroblastoma-spinal cord (NSC) hybrid cell colonies were cloned by pipette selection and limiting dilution at 2-4 weeks in culture. One spontaneous HPRT revertant was recovered from multiple platings of 1×10^8 N18TG2 cells in HAT medium; the hybrid colony formation rate (1 to 5 per 1×10^6 N18TG2 cells) exceeded the revertant rate by at least 100-fold. No cell line has developed from 5 primary spinal cord cell HAT cultures. Most NSC clones were morphologically distinct from the N18TG2 parent and ranged from anaplastic round cells proliferating in suspension, to adherent cells extending multiple long slender processes. Of the 26 NSC clones exhibiting this "neuronal" morphology, 5 extended processes that formed stable contacts with myotubes derived from the C2 mouse myoblast line, similar to dbcAMP-induced NG108-15 and NCB-20 cells. Constitutive expression of choline acetyltransferase (ChAT) was observed in 4 of 23 NSC clones, but not in parent N18TG2 cells. ChAT-expressing NSC-34 cells were investigated with current clamp microelectrode techniques. Resting potentials were observed at ∿40mV. Depolarizing action potentials of ∿ 35mV were elicited by anode break, and were followed by long (200-300 mS) depolarizing (2-3mV) after-potentials. NSC lines may provide reagents for dissection of motor neuron cell biology in a clonal system. (Supported by the ALS Association, ALS Society and MDA.)

420.8 HLA-DR SCHWANN CELL REACTIVITY IN HEREDITARY MOTOR AND SENSORY NEUROPATHIES. G.L.Mancardi*,I.De Martini*, A.Cadoni*, A.Zicca*,D.Bianchini*, M.Tabaton*, A.Schenone* (SPON: E. Mancall). Institute of Neurology and Human Anatomy, University of Genova, via De Toni 5, 16132 Genova, Italy.

T cell recognition of HLA-DR molecules on antigen presenting cells is a critical step of the initial phase of the immune reaction. Apparently only a few HLA-DR bearing cells are able to induce and control the immune response. HLA-DR antigens are expressed only on B lymphocytes, activated T lymphocytes, macrophages, dendritic, endothelial and other somatic or tumoral cells.Recently HLA antigens have been found on Schwann cells in peripheral neuropathies but not in normal control cases(Cadoni et al,Lancet ii:1282,1986;Pollard et al,J Neuroimmunol 13:123,1986;Wekerle et al, Eur J Immunol 16:1551,1986). The aim of the present report is to add new data on the HLA-DR Schwann cell reactivity in hereditary degenerative diseases of the peripheral nervous system.Mouse monoclonal antibodies anti Leu HLA DR,Leu M1,Leu M2,Leu M3,Leu 7, OKM1,OKIa,OKT4,OKT6,OKT8,S-100 were incubated with frozen sections of sural nerve biopsies of patients with hereditary motor and sensory neuropathy (HMSN).The sections were processed according to the peroxidase antiperoxidase method and the immunoreactivity was detected with diaminobenzydine. Schwann cells were cultured between two glass coverslips from sural nerve biopsies of patients with HMSN.This tecqnique yelds a fairly great amount of Schwann cells due to the firm attachement of all the explants.Schwann cells were incubated with rabbit anti human S-100 and mouse anti human DR antiserum and the reactivity detected with goat anti rabbit fluorescein conjugated and goat anti mo use rhodamine conjugated antiserum.In frozen sural nerve sections all the Schwann cells were strongly HLA-DR positive.The whorls of the onion bulbs were stained by the an ti HLA-DR and anti S-100 antiserum. The Schwann cells were also HLA-DR and S-100 positive in the first few days of culture. After 25 days almost 50% of the Schwann cells were still HLA-DR positive.

The finding of HLA-DR positive Schwann cells seems to suggest that, in HMSN, the appearance of Class II histocompatibility antigens is a generic and unspecific sign of cellular activation. We should however consider also the possibility that immune mediated cellular mechanisms could have a pathogenetic role in activating and maintaining the chronic degenerative process in HMSN.

420.9 IMMUNOHISTOCHEMICAL AND IMMUNOFLUORESCENCE LOCALIZATION OF ANGIOTENSINOGEN AND ANGIOTENSIN PEPTIDES IN PRIMARY FETAL RAT BRAIN CELL CULTURES. V.G.Kalter* and M.P. Printz. Dept. of Pharmacology M-036, Univ. Cal. San Diego, La Jolla, CA. 92093.

While the existence of an endogenous brain angiotensin system (BAS) is no longer questioned, the specific cell type(s) which contain the angiotensin (ANG) peptides and/or angiotensinogen (ANGogen), the putative prohormone, are still controversial. To study the BAS at the cellular level we have established and maintained primary fetal rat brain cell cultures under serum-free conditions. The cell populations contain specific binding sites for the angiotensin peptides and secrete ANGogen into the medium. We have used cell specific markers to identify neurons and glia and antisera specific for angiotensins I (ANG I), II (ANG II) and ANGogen in co-localization studies. We used the avidin-biotin peroxidase (ABC-PO) method and the avidin-biotin alkaline phosphatase (ABC-AP) method as well as immunofluorescence on nonconfluent cultures. Cells were first stained for components of the angiotensin system and then with cell specific antisera. Using ABC-PO and ABC-AP immunohistochemistry we have found that ANGogen localizes primarily to neurons with however, some glia exhibiting specific immunoreactivity. Angiotensin peptides are found almost solely in neurons with few if any glia staining for the peptides. These results were confirmed in preliminary experiments using indirect immunofluorescence with either fluorescein-labelled or rhodamine-labelled secondary antibodies. While these serum-free cultures consist of a mixed population of cells, approximately 50-60% are neuronal. Of these, a suprising 20-30% appear to contain immunoreactive ANGogen while a smaller percent contain ANG peptides.

We conclude that in serum-free cultures of primary rat brain cells both the prohromone and peptide are localized largely in neuronal cells while the prohormone may also be contained in a subpopulation of glia. The latter may reflect uptake rather than de novo intracellular synthesis. This cellular system will be valuable for studies of regulation of the brain angiotensin system. (Supported by AHA California Affiliate #86-511 and HL 35018.)

420.10 CALCIUM TRANSFER ACROSS THE ENDONEURIAL CAPILLARIES OF FROG SCIATIC NERVE. H. Levitan, K.C. Wadhwani and S.I. Rapoport. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

In view of the critical role that Ca plays in modulating many functions of vertebrate peripheral nerve, we studied properties of the Ca transfer across the endoneurial capillaries of female frogs, R. pipiens. An *in situ* perfusion technique and an *in vivo* i.v. bolus injection technique were used. For *in situ* perfusion, the vasculature of the hindquarters of an anesthetized frog was perfused with Ringers solution containing ^{45}Ca, and ^{3}H-inulin was used as a vascular marker. After perfusing for 5, 10 or 20 min, the sciatic nerve was exposed and desheathed. For the *in vivo* i.v. bolus injection technique, Ringers (0.2 ml) containing ^{45}Ca and ^{3}H-inulin was injected as a bolus into a left cutaneous vein. After 5 min, the hindquarters of the animal were severed from the body. In both techniques, the permeability-surface area product (PA) of ^{45}Ca at endoneurial blood vessels was calculated from the amount of radioactivity in the desheathed segment, corrected for the contribution of radioactivity via the perineurial route. The PA of ^{45}Ca, calculated from both techniques, equaled 4.4×10^{-5} $ml.sec^{-1}.g^{-1}$ wt wt, and was independent of the $[Ca^{2+}]$ in the perfusate between 0.18 and 18 mM. The half time ($t_{1/2}$) for endoneurial Ca to equilibrate with plasma Ca was calculated to be about 75 min. The low, concentration-independent permeability of the endoneurial capillaries of frog sciatic nerve to Ca probably limits significant rapid changes in the Ca content of the nerve endoneurium during transient changes of plasma Ca, but does not alter steady-state responses. The results are consistent with the absence of homeostasis of whole nerve Ca during chronic hypo- and hypercalcemia (Wadhwani et al., Soc. Neurosc. Abstr. 12:1260, 1986).

420.11 STRUCTURAL AND FUNCTIONAL CONSEQUENCES OF PC12/ADRENAL MEDULLARY ENDOTHELIAL CELL INTERACTIONS THAT ARE MEDIATED BY SPECIFIC CELL SURFACE PROTEINS.
A.Munoz*, Y. Mizrachi*, D. Tombaccini*, R. Ornberg*, H.B. Pollard and P.I. Lelkes* (SPON: K.L.Kelner), Lab. Cell Biol. & Gen., NIDDK, N.I.H., Bethesda, MD, 20892

Adrenal chromaffin cells, a common model for studying neurosecretion, are *in situ* juxtaposed to capillary endothelial cells. Interactions between these two cell types might therefore be of importance for regulating the secretory functions. We have previously shown (*J.Cell Biol. 103, 232a*) that *in vitro* PC12 cells, an adrenomedullary tumor cell line, specifically adhere to capillary endothelial cells, isolated from bovine adrenal medulla (BAME cells). We have further characterized these interactions and report here on their structural and functional consequences. PC12/endothelial cell adhesion was insensitive to calcium levels above 1 mM in the growth medium and inhibited by serum proteins. Adherence of PC12 cells to endothelial cell monolayers (grown in 24 well plates) was independent of the cell density, provided more than $7x10^4$ PC12 cells/ml were used.

Within one hour following plating onto BAME cells, PC12 cells were found to release augmented levels of [^{3}H]-norepinephrine upon stimulation with either acetylcholine, potassium or barium. PC12 and BAME cells in co-culture mutually inhibited their respective growth rates. PC12 cells, grown on endothelial cell monolayers, also lost their responsiveness to nerve growth factor. This is consistent with our previous suggestion, based on the elevation in the met-enkephalin contents in PC12 cells, that upon co-culture with the BAME cells, PC12 cells might reroute their differentiation towards a more chromaffin-like state.

PC12/BAME cell adhesion was selectively inhibited by trypsin sensitive proteins residing in proteoliposomes derived from cell surface extracts. These extracts, containing enriched amounts of a small fraction of plasma membrane proteins, were used to raise polyclonal antibodies. The purified IgG fraction specifically bound to membranes of PC12 cells and endothelial cells, respectively. Preliminary data also indicate that the anti-BAME-extract antibodies recognize one single ~ 62 kD protein. By contrast the antibodies raised against PC12 extracts reacted with several PC12 cell surface proteins. Currently we are characterizing those molecules that mediate specific interactions between PC12 and endothelial cells.

421.1 FUNCTIONAL DIVERSITY OF MULTIPLE VARIANTS OF THE HEAVY NEUROFILAMENT PROTEIN SUBUNIT (NFP-H) IN RETINAL GANGLION CELL AXONS. S.E. Lewis* and R.A. Nixon. McLean Hosp., Harvard Med. Sch., Belmont, MA 02178

Recent studies suggest that the axonal cytoskeleton has a more complex organization than previously recognized. The existence of a stationary non-uniform cytoskeleton as well as a moving component (Nixon and Logvinenko, J. Cell Biol. 102:647, 1986) implies that the interaction of cytoskeletal elements during axoplasmic transport must be highly dynamic and tightly regulated. Post-translational modifications of NFPs could coordinate these interactions by altering the affinity of neurofilaments (NFs) for cytoskeletal structures. Since NFP-H has been implicated as a possible cross-bridge between cytoskeletal elements, its modification may be particularly relevant. Recently, a relatively unmodified NFP-H has been found exclusively in perikarya, while a modified NFP-H is predominantly axonal. We now report that at least four NFP-H variants are present within the axons of a single neuron type. These isoforms are generated by phosphorylation and appear to serve different functions.

Proteins in RGC neurons were selectively labeled by injecting mice intravitreally with ^{35}S-methionine or ^{32}P-orthophosphate. At various post-injection intervals, the optic nerve tracts containing labeled proteins were analyzed by high-resolution SDS-PAGE (3-7% polyacrylamide gradient) and autoradiography. Four proteins in the 195-210 Kd region of SDS gels were identified as NFP-H variants on the basis that each was Triton-insoluble, phosphorylated in vivo, conveyed exclusively in the slowest phase of axoplasmic transport, recognized by antibodies to intermediate filaments and NFP-H. The four variants exhibited nearly identical 2-D chymotryptic iodopeptide maps. Phosphorylation is the likely basis of NFP-H microheterogeneity. NFP-H was synthesized as a single 160 Kd protein that incorporated ^{32}P concomitantly with appearance of NFP-H variants in axons. When enzymatically dephosphorylated in vitro, the four variants were converted to a single 160 Kd band. In vivo labeling studies indicated that the lower MW forms incorporated less phosphate than the higher MW forms. The NFP-H variants distributed non-uniformly along RGC axons. The 197 Kd form predominated in proximal axons, while the two highest MW variants became increasingly prominent at more distal axonal levels, paralleling the distribution of the stationary NF network. The same two variants, pulse-labeled in vivo, were preferentially retained in axons, presumably in association with the stationary cytoskeleton, while the 197 Kd form was exclusively associated with the moving component. A monoclonal antibody recognizing a phosphate-dependent site on NFP-H that appears late in axonal maturation selectively recognized the two NFP-H variants predominating in the stationary cytoskeleton. We propose that phosphorylation regulates the specific association of NFs with different functional domains of the axonal cytoskeleton. AG02126, AG05604, A. and S. Gitenstein Foundation.

421.2 NON-COHERENT AXOPLASMIC TRANSPORT OF TUBULIN AND A HIGH MOLECULAR WEIGHT MAP. R.A. Nixon, I. Fischer*, S.E. Lewis* and L.I. Binder (SPON: T.O. Fox). McLean Hospital, E.K. Shriver Center, Harvard Med. Sch., Belmont, MA 02178; U. of Alabama, Birmingham, AL 35294.

Microtubule-associated proteins (MAPs) play an important role in regulating microtubule function and interactions between microtubules and other cytoskeletal organelles. Tau proteins have been previously shown to be transported in axons at the same rate as tubulin, implying a tight structural association between these proteins. Although specific high molecular weight MAPs have been localized to axons by immunocytochemistry, their structural and functional relationships to microtubules are not well understood. In this study we investigated the transport, posttranslational modification and turnover of a major high molecular weight MAP in retinal ganglion cell (RGC) neurons. Proteins in RGC neurons were selectively labeled by injecting mice intravitreally with ^{35}S-methionine or ^{32}P-orthophoshate. Proteins in consecutive segments of the optic nerve and tract from these animals were analyzed by SDS-PAGE (3-7% polyacrylamide gradients) and autoradiography. A relatively prominent protein of approximately 340 kD was identified as a MAP on the basis of its co-assembly with mouse microtubules during two cycles of assembly and disassembly. In addition, the protein co-migrated with MAP 1 from brain and was selectively recognized by monoclonal antibodies against MAP 1b but not by antibodies against MAP 2. The polypeptide incorporated ^{32}P when RGC neurons were radiolabeled in vivo with ^{32}P-orthophosphate. After intravitreal injection of ^{3}H-proline, the wavefront of radiolabeled MAP 1 progressed through consecutive optic pathway segments. Linear regression analysis of the wavefront for six post-injection intervals between 1 and 45 days revealed a transport rate of 1.5-2 mm per day. Further kinetic studies demonstrated that the disappearance of MAP 1 was biphasic. The first pool disappeared exponentially between 6 and 30 days with a half-life of 11-12 days, indicating that this loss represented the exit from axons of MAP 1 associated with the SCb or Group IV phase of axoplasmic transport. About 15% of the total pool of radiolabeled MAP 1, however, remained in axons after 45 days and disappeared exponentially at a much slower rate (half-life = 55 days). This pool, representing 40-50% of the total MAP 1 content in the steady state, displays the same turnover rate as neurofilament proteins in the stationary cytoskeleton of RGC axons. We interpret these data to indicate that MAP 1 is transported in advance of major structures with which it forms functional associations and that it may participate in the integration of microtubules and other cytoskeletal elements into a stationary network along the axon. AG02126, AG05604, Anna and Seymour Gitenstein Foundation, Inc.

421.3 MONOCLONAL ANTIBODY G2 RECOGNIZES AN EPITOPE OF SEVERAL RAT INTERMEDIATE FILAMENTS AND OF A NON-INTERMEDIATE FILAMENT PROTEIN. C.E.Miller, J.M.Gossels and V.M.Ingram. Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Intermediate filaments comprise one of three major fiber systems of the animal cell cytoskeleton. Their constituent proteins can be defined biochemically, immunologically and by cell type specificity. Their roles in cellular functions, including motility, process extension, axonal transport, secretion and cell structure are unknown. The monoclonal antibody G2 recognizes an epitope of intermediate filaments in several cell types.

G2 was raised against an E16 rat forebrain membrane preparation, enriched in growth cones, which was made according to Pfenninger, et. al.(Cell 35:573, 1983). It was selected by indirect immunofluorescent staining of NB2A neuroblastoma cells. G2 stains non-neuronal cells in primary cultures from E18 total rat brain. Non-neuronal and some neuronal cells stain in P5 rat cerebellum primary cultures. Heart and kidney cells from P5 rat primary cultures also stain. In all cells, the staining resembles the pattern characteristic for intermediate filaments. The filaments are often predominantly located in the perinuclear region, but they extend throughout the cell. In astrocytes, the staining pattern is quite similar to that obtained with antibodies to GFAP. G2 stains processes and some cell bodies in E17, E18 and P1 rat brain sections.

Immunoblot analysis of newborn rat brain, heart, liver and kidney homogenates indicates that G2 recognizes a 200kD protein. This protein is enriched in the growth cone preparation. The 200kD protein is solubilized by Triton-X100 and, at least in heart cells, probably represents myosin. G2 also recognizes proteins of m.w. 69kD, 62kD and 58kD, which are greatly enriched in the growth cone preparation. These proteins are Triton-X100-insoluble, and they probably include the smallest neurofilament protein and vimentin.

G2 recognizes an epitope that we believe is common to several but not all intermediate filament proteins. It also recognizes a protein that is not an intermediate filament protein. We are continuing our characterization of all these proteins and their relationships to each other. This antibody should be a useful probe for the structure and functions of intermediate filaments in many cell types, especially those of the nervous system.

421.4 NEUROFILAMENTS IN CRAYFISH AXOPLASM. S.A.Halls*, R.A.Sheller and G.D.Bittner, Institute of Neurological Sciences and Department of Zoology, University of Texas at Austin, Austin, Texas 78712.

Arthropod axons have often been reported to lack neurofilaments (NF). However, the CNS of crayfish contains calpains (EC 3.4.22.17), which are calcium requiring enzymes that degrade NF's and that are active at μM calcium concentrations (Nosek & Crosland, *J. Neurobiol.* **14**, 9-16, 1983). Since most previous studies have relied upon electron microscopy, the use of extracellular buffers and fixatives that have supraphysiological levels of calcium (compared to normal intracellular levels) may have degraded NF's.

To investigate if NF's are present in crayfish, axoplasm collected from CNS neurons was subjected to SDS-PAG electrophoresis and then transferred to a nitrocellulose membrane. The nitrocellulose membrane was wetted with transfer buffer (25mM Tris, 192mM glycine/20% (vol/vol) methanol at pH 8.3) and assembled in a cassette and placed into a mini-transphor apparatus which had been filled with transfer buffer. Transfer of the proteins was carried out at 200mA for 3 hours; the membrane was prepared for immuno-detection of NF-like proteins.

The electrophoretic blots (unstained) were transferred to a vessel containing 5% BSA in 0.9% NaCl/10mM Tris-HCl, pH 7.4 overnight at 4°C to saturate non-specific protein binding sites. The nitrocellulose membrane was washed three times in PBS and incubated with antisera directed against NF's. The primary (1/2000) and peroxidase labeled secondary antibodies (1/200) were diluted in 1% NGS in PBS. The membrane was placed in an incubation trough, completely overlain with the primary antisera, and incubated at 37°C for 2 hours. The strips were washed four times for 15 min each with 50ml of PBS containing 1% NGS and 0.05% Tween 20. The membrane was then incubated in the secondary antibody for 1 hour at 37°C and washed as described above. The peroxidase substrate reaction was performed by a 10 minutes incubation of the strips in 3-amino-9-ethylcarbazole in dimethylformamide to which 0.05M acetate buffer (pH 5.0) had been added. A red insoluble precipitate formed at sites of antigen-antibody complex.

Electrophoretic and Western transfer analyses of crayfish axoplasm reveal NF-like proteins that have electrophoretic and immunological properties similar to those of the 200kd and 160kd species of NF proteins found in vertebrates. We did not find NF-like proteins in crayfish that are recognized by a monoclonal antibody directed against the 68kd mammalian NF protein. The absence of the 68kd species in the axons of these crayfish may be due to (i) the inability of the antibody to recognize a similar epitope in crayfish as it does in mammals, or (ii) the amount of antigen present in crayfish is below the limit of detection by our method. Electrophoresis of glial sheath homogenate shows very little protein corresponding to 200kd or 160kd. To check for glial contamination of the axoplasm, an antibody to GFAP was used on axoplasm. No immunostaining bands were seen.

Our data provide biochemical and immunological evidence that there are NF-like proteins in an arthropod species and eliminates a presumed evolutionary anomaly in arthropods which have been thought to lack such structures (Lasek et al. *Annals N.Y. Acad. Sci.*. **455**, 462-478, 1985).

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421.5 **ACTIVATION OF RAT BLOOD PLATELETS PRODUCES A CALPAIN-MEDIATED DEGRADATION OF A BRAIN SPECTRIN-LIKE PROTEIN.** K. Kramer*, P. Seubert*, G. Lynch, and M. Baudry. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

When stimulated by a variety of agonists, blood platelets exhibit a variety of structural modifications affecting the cell cytoskeleton and the distribution of cell surface receptors. We previously proposed that a variant of this underlying mechanism operates in brain dendritic spines and is responsible for modification in synaptic structure and functions elicited by brief periods of high frequency stimulation. In particular we suggested that activation of a calcium-dependent protease (calpain) and the resulting degradation of fodrin in brain spectrin was a general mechanism by which cells modify their structures and functions in response to external stimuli. In the present study we used a polyclonal antibody against brain spectrin to investigate the existence of the calpain/spectrin interaction in blood platelets.

Rat blood platelets exhibit a brain spectrin-like protein which migrates on SDS-polyamylamide gels between the actin-binding protein and P235, and appears as a doublet protein with an apparent Mr of 245 and 240 Kd. This protein is almost entirely associated with the Triton-insoluble fraction and thus is part of the platelet cytoskeleton. Platelet spectrin-like protein is degraded by purified calpain generating a major breakdown product with an Mr of 150 Kd. The polypeptide map produced by calpain from platelet spectrin is virtually identical to that from rat brain. Moreover for both platelet and rat brain, calmodulin increases the rate of proteolysis and produces additional degradation products. Platelets contain calpain and endogenous platelet calpain degrades platelet spectrin in a similar fashion as purified calpain. When intact platelets are activated by a number of stimuli, a fraction of platelet spectrin is degraded as evidenced by the increase in the 150 Kd breakdown product. The calpain inhibitor, leupeptin, prevents both the thrombin-induced platelet activation and the degradation of platelet spectrin.

Our results indicate that platelets exhibit a spectrin-like protein which shares a number of properties with brain spectrin and that activation of platelets produces a calpain-mediated proteolysis of spectrin. This model should provide useful information concerning the role of spectrin and its proteolysis in the regulation of cell structure. (Supported by grant BNS 81-12156 to MB and AFOSR #86-0099 to GL.)

421.6 ELUCIDATION OF THE ROLE OF CALCIUM IN THE ELECTRIC ORGAN OF ELECTROPHORUS ELECTRICUS. M.A. Kaetzel*, R.R. Scully* and J.R. Dedman*, (SPON: R. Glantz). Department of Physiology and Cell Biology, The University of Texas Medical School, Houston, Texas 77025.

Calcium is an important regulator of neuromuscular function. Calmodulin is the major intracellular mediator of calcium action. Calmodulin binding proteins, few of whose enzymic functions are known, are the targets of active calcium-calmodulin. These proteins convert the calcium signal into coordinated physiological responses such as cyclic nucleotide metabolism and secretion. In order to identify calmodulin-dependent pathways involved in neurotransmission, those which have remained undetected by previous methodologies, we have utilized the E. electricus electric organ. Our approach has been to purify and characterize calmodulin associated proteins from this calmodulin and synapse rich tissue. Calmodulin is a major protein in the electric organ. Localization studies using the PAP technique demonstrates that calmodulin is present in the electrocyte and associated nervous tissue of the electric organ. We have employed calcium dependent hydrophobic chromatography to investigate the presence of other calcium mediator proteins including the calcimedins. Calcimedin 67 kDa is expressed in the electric organ.

In order to evaluate the discrimination of the calcium signal through calmodulin we have utilized calmodulin-Sepharose chromatography to identify calmodulin target proteins. There are approximately twenty polypeptides from the electric organ which bind calmodulin in a calcium dependent manner. Two protein species, 55 kDa and 47 kDa, are the most abundant and possess the highest affinities for calmodulin. Each protein was purified and antibodies produced. Immunoblot analysis of fresh tissue revealed only the 55 kDa species. The 47 kDa species was shown to be generated by endogenous proteolysis during purification. The 55 kDa calmodulin protein is also present in skeletal muscle but not in non-excitable tissues. A distinct, immunocrossreactive calmodulin binding protein was identified in the electric organ, heart, brain and spinal cord.

It is anticipated that these calmodulin target proteins play subtle, yet essential, roles in neurotransmission. Immunolocalization of these proteins following physiological manipulations may provide further insight into the molecular aspects of the role of calcium in the regulation of bioelectricity. (Supported by GM29323).

421.7 HORMONE- AND TRANSMITTER-ACTIVATED CHANGES IN INTRACELLULAR CALCIUM CONCENTRATION; A SIGNAL TO IDENTIFY AND PURIFY SUBPOPULATIONS OF CELLS. I. Schieren* and A.B. MacDermott. Howard Hughes Medical Inst., Columbia Univ. CPS, New York, NY 10032.

Investigation of the physiological and chemosensitive properties of developing neurons in tissue culture is hampered by the heterogeneity of cell types. In the absence of selective antibodies or distinctive morphology, it is difficult to identify subpopulations of neurons. A functional distinction between neurons may be made, however, on the basis of differential transmitter receptor expression. Response to transmitters, peptides and hormones in many cases involves an increase in $[Ca^{++}]_i$. We have therefore developed a method for cell identification and separation that combines the selectivity of the ligand/receptor interaction, the calcium indicator INDO-1 and a fluorescence activated cell sorter to analyze cells based on an increase in $[Ca^{++}]_i$ after agonist binding.

The EPICS 753 cell sorter was modified with a dual-flow mixing system that permits the cells and agonist to mix at a fixed time 1-30 seconds before the cells enter the flow tip. Since the shift in $[Ca^{++}]_i$ following activation can be transient, it was important to have a constant time between exposure to agonist and fluorescence detection so that data could be collected and sorting pulses generated for each cell at the time of peak change in $[Ca^{++}]_i$. Fluorescence was measured at two different emitting wavelengths (400 & 490 nm) and expressed as a ratio. Cell sorting windows were set to collect viable cells with a shift in their ratio fluorescence. The pancreatic acinar cell line AR4-2J and an acinar-derived cell line AR4-1P were used to develop this system since the AR4-2J cells respond to the peptide Substance P (SP) with an increase in $[Ca^{++}]_i$ (Womack et al, 1985) while the AR4-1P cells do not. Purification was optimized by mixing the AR4-2J and AR4-1P cells in known proportions and comparing the number of cells responding to SP with the number of AR4-2J cells in the mixture. There was a close correspondence between the percent of AR4-2J cells added to the mixture and the percent of cells responding to SP. These cells were sorted, placed in culture overnight and stained with an AR4-2J selective antibody to test the purity of the sort. More than 95% of the cells that responded to SP showed antibody staining while less than 5% of the nonresponders showed any staining.

We have used this technique to follow the developmental appearance of peptide and excitatory amino acid receptors in acutely dissociated embryonic and newborn rat spinal cord neurons. We have been able to show changes in the percentages of cells responding to transmitters at different times in development and have sorted and grown cultures of neurons identified by agonist activation.

421.8 SELF-ASSEMBLY IN VITRO OF INDIVIDUAL NEUROFILAMENT SUBUNITS. J.C. Troncoso†, M. Häner*§, D.L. Price† and U. Aebi*§°. †Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; §Univ. of Basel, Biozentrum, Maurice E. Müller Institute; °Dept. of Cell Biology, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The present study was designed to investigate the self-assembly in vitro of the three neurofilament (NF) subunits (210 kD [H], 160 kD [M], and 68 kD [L]). NFs from bovine spinal cord were solubilized in 6M urea-100 mM MES buffer and individual polypeptides separated by anion exchange chromatography. Subsequently, each NF subunit was equilibrated against urea-free 100 mM MES buffer, 170 mM NaCl, 0.5 mM EGTA, 1 mM DTT, pH 6.5 at 37°C for at least three hours. Filament assembly was monitored by electron microscopy of negatively stained dialysate specimens. The L protein formed long, flexible, and smooth 10-nm filaments that were undistinguishable from other intermediate filaments (IF); this process was enhanced by Mg^{++} (1-10 mM). The M and H proteins reassembled into short, S-shaped, polymorphic 8-12 nm filaments. Equilibration of the L protein against buffer at pH 5.0 or at 4°C led to reconstitution of short, irregular 10-nm filaments that did not elongate further upon subsequent equilibration at standard conditions. Treatment of reconstituted NF L with 10 mM Na phosphate produced a striking unraveling into protofibrils and protofilaments similar to those that occur with similar treatment of keratin filaments. Determination of mass-per-unit length of the three types of reconstituted NF is in progress.

Features of in vitro assembly of the L subunit of NFs, including morphology and unraveling pattern into subfilamental structures, resemble those occurring with other IF. The M and H subunits self-assemble into distinct filaments, morphologically different from other IF. Temperature and pH are critical factors for self-assembly, and changes in these parameters lead to formation of aberrant filamentous structures that cannot be rescued by equilibration at standard conditions.

421.9 AXONAL TRANSPORT OF NEURONAL ANTIGENS CHARACTERISTIC OF SUBPOPULATIONS OF CNS NEURONS. R.D. Atkinson*, C.A. Miller* and J.A. Garner. Departments of Anatomy and Neuropathology, USC Sch. of Med., Los Angeles, CA 90033.

Certain monoclonal antibodies (MAbs) identify nervous system antigens that are localized to neuronal subpopulations and that may undergo change during the course of neurodegenerative diseases, such as Alzheimer's Disease (Miller, et al. Soc. for Neurosci. 12:100a, 1986). We have now examined the axonal transport of 4 of the corresponding antigens for two reasons. First, neuronal forms of the antigens can be unequivocably identified by their axonal transport. Second, each of the three major axonal transport rate-components is thought to convey a subcellular structure within the axon: the fast component (FC) provides membranes and membrane proteins, while the 2 slow components (SCb, SCa) convey the cytoplasmic matrix and cytoskeleton, respectively. Association of an antigen with a particular rate-component may give insight into its intracellular functions and its structural nature.

Radioactive amino acids were unilaterally injected into the vitreous of guinea pig eyes. Radiolabeled proteins synthesized in retinal ganglion cells were axonally transported into the optic nerve and tract. After appropriate intervals permitting separation of the rate-components in the axons due to their unique transport rates, animals were sacrificed. Optic nerve containing radioactive proteins characteristic of single rate-components, and contralateral control nerves, were removed and subjected to immunoadsorption with each of the four MAbs. Visualization of radiolabeled antigen was by gel electrophoresis followed by fluorography.

All four MAbs identified antigens specifically associated with single axonal transport rate-components. Three MAbs (3F12, 44.1, and 8C3) specifically identified polypeptide bands from tissue containing radioactive SCb proteins. Another MAb (22C10) identified 2 polypeptide bands from tissue which contained radioactive SCa proteins. None of the MAbs identified antigens from tissue containing radioactive FC proteins.

Assignment of these antigens or antigen subunits to specific subcellular systems has permitted their characterization as putative cytoplasmic matrix proteins (3F12, 44.1, 8C3) or cytoskeletal proteins (22C10). The roles these antigens may play in the nervous system during normal axonal function and during neuropathogenesis can now be further examined. This work was supported by NIH Grants NS22402 and MH39145.

421.10 **MODULATION OF RENAL PORE ACTIVITY BY L10 AND THE LUQ CELLS OF APLYSIA.** **J. Koester, M. Chen, & C.H. Bailey. Center for Neurobiology & Behavior & Dept. of Psychiatry, Columbia University & N.Y. State Psychiatric Institute, N.Y., NY 10032**

Although the abdominal ganglion of Aplysia has long been popular for studying the neurobiology of behavior, the behavioral functions of most of the larger cells in the ganglion remain unknown. Here we show that some of the cells used most extensively for cellular studies are involved in the control of renal function.

Renal excretion in Aplysia begins with the formation of an ultrafiltrate of blood across the wall of the heart. This fluid is swept from the pericardium into the renal sac through a cilia-lined duct. Within the renal sac the fluid is further elaborated into urine by excretion and absorption across the epithelial sheet of the renal sac. Urine leaves the renal sac via the muscular renal pore located near the posterior base of the gill (Eales, 1921).

Neurons L10 and the 5 left-upper quadrant (LUQ) cells, L2-L6, are located in the abdominal ganglion. L10 has been shown by others to be cholinergic. More recently it has also been found to contain the neuropeptide myomodulin (Alevizos, et al., this volume). The function and the transmitter type of the LUQ cells have not been described, although neuron L5 has been shown to express a gene that encodes a product with the characteristics of a neuropeptide (Shyamala, et al., *DNA*, 1986). Both L10 and cells L2,L3,L4 and L6 have been shown to possess endogenous bursting properties, and L10 is known to inhibit the LUQs.

These neurons seemed to be likely candidates to modulate renal function because: (1) L10 had been shown to increase heart rate (by exciting heart excitor cell RB-HE), so it may increase the rate of renal filtration; (2) We have found that L10 and a sub-set of the LUQs send axons to the renal pore.

Four types of evidence support the hypothesis that these cells control pore opening: (1) Hyperpolarizing L10 by current injection to eliminate spontaneous firing reduces the frequency of pore opening from 58/hr to 2/hr (N=6; reduced preparation); (2) Firing L10 by current injection initiates pore opening and firing some of the LUQ cells initiates pore closing; (3) L10 spikes elicit 1:1 IJPs in the spiking circular muscle that causes pore closure; (4) Touching the pore triggers pore closure and inhibits L10.

To explore further the role of L10 and the LUQs in controlling pore activity, we have begun an ultrastructural survey of synaptic contacts on circular and radial muscle fibers that control pore opening. Preliminary quantitative analysis indicates that at least three general types of neuromuscular junctions can be distinguished, based on vesicle morphology and contact location. In addition, small islands of axonal profiles form what appears to be part of a peripheral neural plexus containing numerous synaptic contacts. This plexus may form an anatomical substrate for presynaptic modulation of transmitter release onto the pore muscle.

421.11 BRAIN ORGANIZATION OF MANDUCA SEXTA STUDIED WITH MONOCLONAL ANTIBODIES

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Monoclonal antibodies (mAbs) directed against the central nervous system of Manduca sexta have been isolated by screening 1,105 hybridoma cell lines prepared by using mice immunized with tissue of the primary olfactory center (Hishinuma, A. et al., 1985 Soc. Neurosci. Abstr. 11:919, and Hishinuma, A. 1986 Ph.D. Dissertation, Columbia Univ.). As part of our continuing studies of cell differentiation and organization of Manduca brain, we examined the complete distribution of the antigens recognized by three of these antibodies. 40-µm frozen sections were reacted with hybridoma culture supernatants and subsequently rabbit anti-mouse IgG conjugated to HRP, and reaction product was developed by DAB under hydrogen peroxide.

The immunoreactivity of mAb 1C2 is confined, except for a few large cells in the subesophageal ganglion and protocerebrum, to most or all brain areas in which synaptic structures can be identified-- for example, glomeruli in the antennal lobe, the lamina, medulla, lobula, and lobula plate in the optic lobe, the calyces and α-, β-, γ-, and γ lobes of the mushroom body, and the central body complex. No fiber tracts are stained. In electron-microscopic immunocytochemistry the reaction product is found on plasma membrane and internal membrane structures, including synaptic vesicles, in a subpopulation of synapses in the antennal lobe.

MAb 22E11 stains clusters of small cells (but larger than Kenyon cells), located around the calyx of the mushroom body and, much less intensely, a few large cells in the protocerebrum, antennal lobe, and subesophageal ganglion. Calyces, peduncle, and α-, β-, γ-, and γ lobes of the mushroom body and the central bodies are distinctively stained among most or all synaptic areas elsewhere, which are faintly stained.

MAb 28H2 has been described as a Glia-Specific Antibody that recognizes microtubules in a subpopulation of glial cells in the olfactory pathway (Hishinuma, A. 1986). 28H2 immunoreactivity is identified mainly in fiber tracts where a dense population of glial cells might be found, for example the optic chiasma in the optic lobe, interconnecting area between the optic lobe and protocerebrum, and central neuropil in the antennal lobe. Less intense immunoreactivity is found around neuronal somata in the brain. Neuronal somata themselves are not immunoreactive.

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421PO **BAG CELL AND ATRIAL GLAND GRANULE- AND TISSUE-SPECIFIC ANTIGENS: IDENTIFICATION AND CHARACTERIZATION**

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The bag cell neurons and exocrine atrial gland of the marine mollusc Aplysia californica express tissue specific neuropeptide genes. The peptide precursors encoded by these genes are approximately 90% homologous; however, distinct bioactive peptides are released from the bag cells and atrial gland. These peptides are involved in mediation of the egg-laying fixed action pattern.

ELH (egg-laying hormone) and α-BCP (α-bag cell peptide) are active peptides encoded by the gene expressed specifically in the bag cells. Analysis of immunogold labeling with antisera raised against either native ELH or synthetic -BCP demonstrates that relatively more ELH immunoreactivity resides in a smaller class (80-120 nm) of dense core vesicles (DCV) whereas α-BCP immunoreactivity is more intense in a larger class (300-800 nm) of DCV. We have raised monoclonal antibodies (mAbs) to a membrane preparation from the bag cells. Two classes of mAbs were shown to: 1) react specifically with the bag cells; 2) predominantly stain the larger class of DCV and 3) recognize antigens of a different size than the peptide precursor.

The A peptide encoded by the gene expressed specifically in the atrial gland is localized in atrial gland granules that are 1-2 um in diameter. These granules appear to have a complex structure, including a dense core, a less dense outer cortex, and a limiting membrane. A mAb, 5E10, raised against an atrial gland granule preparation stains the cortex, but not the core of the granules. We have used this mAb to immunoprecipitate a protein of MW 57 kD. A serum antibody generated against the immunoprecipitated protein recognizes the same MW band on protein (Western) blots. The subcellular localization indicates that it cannot be an entirely membrane associated protein. We have obtained partial amino acid sequence of affinity purified 5E10 antigen.

These immunological reagents allow further studies of the biogenesis and function of neuroendocrine and exocrine peptides that mediate animal behavior.

422.1 MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERIZATION OF DENTATE HILAR CELLS RECORDED IN GUINEA PIG HIPPOCAMPAL SLICES. H.E. Scharfman and P.A. Schwartzkroin. Department of Neurological Surgery RI-20, University of Washington, Seattle, WA 98195.

We have begun to study a population of cells in the hilus of the dentate gyrus situated between the dentate granule cell layer and the CA3/CA4 cell body layer in the center of the hilus. Intracellular electrodes filled with 1-3% Lucifer yellow were used to morphologically and physiologically characterize 21 cells in 450-475um thick hippocampal slices. Slices were maintained in an interface recording chamber, warmed (35°C), oxygenated (95%O_2,5%CO_2), and perfused with artificial cerebrospinal fluid (in mM: 124 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, and 10 dextrose).

Physiologically these cells were similar to area CA3 pyramidal cells. Depolarizing current pulses produced an accommodating train of action potentials (APs) or a burst, followed by an afterhyperpolarization. I-V curves demonstrated rectification, and the average RMP of these hilar cells (mean±sem= -65.0±2.9mV) was comparable to the RMP for CA3 pyramidal cells. However, input resistance (71.5±6.0 megohms, measured from the response to a -0.2nA, 100 ms current pulse) and time constant (20.5±2.5ms) were greater than CA3 pyramidal cells. There were also more subthreshold spontaneous events seen in the hilar cells than in pyramidal cells.

Stimulation of any area tested (perforant path, stratum moleculare of the dentate, the hilus, str. oriens or radiatum of area CA3, the fimbria, or str. pyramidale of area CA1) elicited mono- or multiphasic depolarizations (presumably EPSPs; up to 20mV, 200ms). In a few cases EPSPs were followed by hyperpolarizations (possibly IPSPs) if the cell was depolarized from the RMP with DC current. Increasing stimulus strength evoked typically one, but in some cases more, orthodromic APs that were followed by depolarizing and hyperpolarizing afterpotentials.

Morphologically many of these cells were similar to mossy cells. Somata were multipolar and cells had thick proximal dendrites that were intensely spiny. Dendrites branched extensively in the hilus and in some cases into the granule cell layer. Axons were relatively large and descended into the hilus, giving off several fine collaterals that branched in all directions, often extending into str. moleculare of the dentate or str. oriens of area CA3/CA4.

It is interesting that these interneurons do not share the physiological characteristics of interneurons of str. pyramidale/oriens of CA1, str. granulosum of the dentate, or the neocortex. Perhaps they are not true interneurons; many hilar cells send projections to the contralateral dentate. Regardless, the physiological and morphological characteristics of these cells suggest that they play an important integrative role in hippocampal circuitry.

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422.2 VISUALIZATION OF HIPPOCAMPAL SYNAPSES IN BRAIN SLICES USING VIDEO MICROSCOPY. T. H. Brown and C. L. Keenan. Div. of Neurosci., Beckman Res. Institute, City of Hope, Duarte, CA 91010.

Recent advances in video microscopy (Inoue, Video Microscopy, 1986) have already had a major impact on cell biology. We have begun to explore the applicability of analog and digital video enhancement techniques to various types of living brain slices, including the lateral geniculate nucleus and visual cortex, the cerebellum, and the hippocampal formation. Our preliminary results suggest that this combination of techniques holds great promise (see also MacVicar, J. Neurosci. Methods 12: 133, 1984).

Here we describe our initial attempt to visualize hippocampal synapses. Hippocampal brain slices (100 - 300 μm thick) were prepared from rats of various ages. The slices were maintained in physiological saline in a small chamber mounted on the stage of an inverted microscope. The region of the chamber that supported the slice consisted of a glass cover slip (140 μm thick) which allowed us to use DIC optics and an oil-immersion objective (N.A. 1.4) for visualization of cytoarchitecture within the hippocampal slices.

Even at low power (10X objective), the regions of the hippocampal slices in which the mossy fiber (MF) synapses are known to be distributed (see Figs. 6 and 7 of Johnston and Brown, J. Neurophysiol. 50: 464, 1983, and Fig. 2 of Brown and Johnston, J. Neurophysiol. 50: 487, 1983) had a distinctly different "texture." In both the stratum lucidum of region CA3 and the infragranular layer of dentate gyrus, there was a prominent zone characterized by a "coarser grain." The postnatal time at which these zones became evident seemed to match the known developmental appearance of the MF synaptic expansions (Amaral and Dent, J. Comp. Neurol. 195: 51, 1981). These coarsely-textured zones were not apparent at 7 - 10 days postnatal but were clear by 14 days and later. Examination of slices from 7, 10, 14, and 22 day-old rats using higher magnification (60X oil immersion objective) revealed what looked like fine axonal processes, but with no MF-like synaptic expansions. However, by 14 days, intermittent spherical or ovoid expansions (2 - 4 μm diameter) could be seen, and the size and numbers of these expansions increased by 22 days.

The size, spatial distribution, and developmental time of appearance of these structures appear to correspond to that described by Amaral and Dent (1981) for the MF synapses of the rat. Using both fluorescent dyes and neurophysiological techniques, we are currently attempting to test more directly the possibility that these visually discernible structures are indeed the MF synapses. (Supported by a McKnight Foundation Development Award and AFOSR contract F49620. We thank Victoria Chang, Xin-Wei Rong, Alan Ganong, Brian MacVicar and Stephen Smith for assistance or useful advice on this project).

422.3 FINE STRUCTURE AND SYNAPTIC CONNECTIONS OF IDENTIFIED NEURONS IN THE MONKEY HIPPOCAMPUS: A COMBINED GOLGI/EM STUDY IN THE BABOON. M.Frotscher, J.Kraft*and U.Zorn*. Inst. of Anat., Univ. of Frankfurt/M., Fed.Rep.Germany.

Two young adult female baboons (papio anubis) were fixed by transcardial perfusion in Nembutal anesthesia for a combined Golgi/electron microscope (EM) study of identified neurons in the hippocampus proper and fascia dentata. After Golgi impregnation, gold-toning, and embedding in Araldite,selected neurons were first subjected to light microscopic study and then serially sectioned for subsequent electron microscopic analysis. Results were compared with previous Golgi/EM studies of hippocampal neurons in small laboratory animals.

Cell bodies of pyramidal neurons in CA1 were more loosely distributed than known from studies on the rat or guinea pig. Numerous basal and horizontal dendrites originating from the perikaryon fill in the space between neighboring cell bodies. Apical stem dendrites are varying in length, depending on the position of the parent cell body in outer or inner portions of the pyramidal layer. Dendrites are densely covered with spines which in the EM show very complex synaptic contacts.

In contrast to our observations in rats, CA3 pyramidal cells in the monkey hippocampus exhibit numerous large spines or excrescences not only on apical dendrites but also on basal dendrites running through stratum oriens. These large spines branch, protrude deeply into presynaptic mossy fiber boutons, and form multiple asymmetric synaptic contacts.

Granule cells of the monkey fascia dentata, in contrast to those of the rodent, often had basal dendrites extending into the hilar region.

Nonpyramidal neurons of the hippocampus proper,which in rodents are usually devoid of spines,showed numerous spines on dendrites and cell bodies. Often one single spine was found to establish synaptic contacts with several presynaptic boutons. In most nonpyramidal neurons characteristic nuclear inclusions were observed.

In conclusion, our correlated light and EM study in the baboon suggests a more complex dendritic pattern and synaptic organization of identified hippocampal neurons in primates than in commonly studied small laboratory animals.

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422.4 INTRACELLULAR INJECTION OF HRP INTO PREGANGLIONIC NEURONS IN THE SACRAL SPINAL CORD OF THE CAT C. Morgan and W.C. deGroat Dept. of Anatomy, UCSF, San Francisco, CA 94143 and Dept. Pharmacology, University of Pittsburgh, Pittsburgh, PA 15261

Preganglionic neurons (PGN) in the sacral spinal cord are important to excretory and sexual function. Our previous studies have examined many aspects of these behaviors, especially the neural morphology. Retrograde HRP tracing has yielded considerable information but because of multiple labeling, details of individual neurons are often obscured. The intracellular injection used in present experiments allows a more precise and complete examination of individual neurons.

Adult cats were anesthetized with dial/urethane (7mg/kg, IP). Following tracheotomy and placement of venous and arterial catheters a laminectomy was performed from the sacrum to T11 and the spinal cord transected at T11 to improve stability. The cats were held in a spinal frame, paralyzed and artificially respired. Sacral ventral roots were transected and stimulated antidromically to determine the location of the PGN through the evoked cord dorsum potential. Bevelled glass electrodes were filled with 4% HRP, resistance 100-140 Megohms. Injections were made with a positive square wave pulse, 4Hz, 200msec, 3-4 nAmps for 3-8 min.

One PGN each was recovered from two of three cats attempted. Both cells were located in lamina VII, one in S3, the other in S2. Their dendrites were free of spines but some fine distal dendrites had varicosities; some were traced over 800-1000 um from the soma. Unlike the lateral band cells reconstructed from retrograde studies which seemed to have four primary dendritic fields; the lateral funiculus, ventrolateral lamnia I, the dorsal commissure, and dendrites within the nucleus itself; these injected neurons had only one type of field each in addition to the dendrites within the nucleus. Thus one PGN projected largely to the dorsal commissure, while the other projected to ventrolateral lamina I. Within each of these fields the five to seven dendrites from each neuron were generally directed caudally and in one case were separated from each other longitudinally by over 1000 um. No axon collaterals were identified.

It is interesting that these neurons were not identical to those described in the earlier, multiple labeling studies as it indicates there may be more variety in the preganglionic neurons in this region than we expected. This new information calls for a reevaluation of our theories of the relationship between the morphology of these PGN and their associated reflexes, particularly micturition.

422.5 SYNAPTIC BOUTON CHANGES ON FACIAL MOTONEURONS FOLLOWING AXOTOMY. S.K. Jacob. Department of Anatomy, Rush Medical College, Chicago, IL 60612.

Facial motor neurons exhibit a transient chromatolysis and no neuronal cell loss following axotomy resulting in a relatively normal morphology by 30 days postoperative (dpo). This study examined axosomatic synaptic bouton contacts on facial motoneurons following severance of the facial nerve with the purpose of further detailing the events during the retrograde response to injury.

Two groups of six adult hamsters underwent axotomy and removal of a distal segment of the right facial nerve. The left nerves remained intact to serve as a control. Brains of each group were perfusion-fixed at 5 or 30 dpo. The facial nuclei were dissected out and further processed for routine ultrastructural examination.

At 5 dpo the neurons showed a morphology consistent with the chromatolytic response. In addition, synaptic bouton contacts were sparse and many thin astrocytic processes ensheathed the neuronal perimeter. At 30 dpo normal neuronal morphology was observed as well as some somal synaptic contacts. Astrocytic processes were still seen intervening between synaptic boutons and the perimeter of the soma. A quantitative study was undertaken to assay the percent coverage of somal profile by synaptic boutons in normal and 30 dpo neurons. Preliminary results show that a normal facial motoneuron has 30% of the perimeter covered by boutons while at 30 dpo only 8.5% of the somal membrane has bouton contacts.

This quantitative study has also shown that the total perimeter (somal membrane) of normal neurons and 30 dpo neurons is the same, confirming that the decrease in percent coverage by synaptic contacts seen at 30 dpo is caused by fewer synaptic boutons present after axotomy. Axotomized facial motoneurons survive the injury even though appropriate peripheral reconnections are not made and synaptic bouton contacts are below normal.

422.6 MORPHOLOGICAL AND ELECTRICAL DIFFERENCES BETWEEN THE DENDRITES OF SINGLE BRAIN STEM MOTONEURONES IN THE CAT. S. Tyc-Dumont, P. Gogan*, H. Bras*, J. Destombes* and A. Barbe*. INSERM U6 and CNRS UA634, 280, bd. Sainte-Marguerite, 13009 Marseille, France.

Laryngeal and abducens motoneurones intracellularly recorded and labelled with HRP were reconstructed in 3D from high resolution measurements using a computer-aided microscope. Each dendrite was isolated by computer dissection and described by the following parameters : spatial projection, length, diameters, tapering, branching pattern, daughter branch ratio and branching power. A principal component analysis applied to the geometrical and the directionnal parameters demonstrated that the individual dendrites of a single motoneurone occupied definite territories and differed from each other by their geometry. In order to retain this multiformity and since the criteria for reducing the arborization to equivalent cylinders models were infringed, cable properties were computed by partitioning the dendrites into a series of contiguous segments short enough to be considered an isopotential cylinder. The steady-state properties of each segment were combined to obtain the following parameters at any site of the dendrite : electrotonic distance, from the soma, input resistance, somatopetal and somatofugal voltage attenuation and charge transfer effectiveness ratio. Individual dendrite showed significant differences in their electrical behaviour. The distal branches displayed low charge transfer efficiency which was not compensated by the size and/or the density of synaptic boutons as revealed by a quantitative ultrastructural analysis at their level.

Low attenuation pathways were revealed between distal branches suggesting the possibility of branch to branch interactions and local computations. Ultrastructural observations of close appositions between labelled dendrites and unlabelled dendrites or somata together with dendro-dendritic synapses suggested that the distal dendrites may be involved in local cell to cell communication.

422.7 ESTIMATING THE ELECTROTONIC STRUCTURE OF NEURONS WHICH CANNOT BE APPROXIMATED AS EQUIVALENT CYLINDERS. W. R. Holmes and W. Rall. Mathematical Research Branch, NIDDK, NIH, Bethesda, MD 20892.

The electrotonic structure of a neuron determines how effective synaptic inputs may be. Formulae for computing the electrotonic length, L, of a neuron (Rall, 1969, 1977) have been widely applied to a number of neuron types. However, these formulae are strictly valid only for neurons which can be approximated as an equivalent cylinder having uniform membrane resistance, Rm (Ω-cm^2). Morphological evidence suggests that some neurons are better represented by two cylinders of unequal length or by a tapering structure. Electrophysiological evidence suggests that Rm may be non-uniform, possibly due to a shunt at the soma produced by electrode penetration. Computations with models which include these complications provide a basis for assessing discrepancies to be expected from naive application of the simple (equiv. cyl.) formulae; they also provide leads for a constrained inverse problem.

The passive voltage decay after a current step can be described as an infinite sum of exponentials $V(t) = \Sigma\ Ci\ \exp(t/\tau i)$. If $\tau 0$, $\tau 1$, C0, and C1 are known for a neuron satisfying equivalent cylinder constraints with uniform Rm, L can be correctly estimated from any of three different experimental ratios: $\tau 0/\tau 1$, C0/V(0), or C0/C1. However, misapplication of the three simple formulae to neurons that are not equivalent to cylinders with uniform Rm yields three different values, labeled L* (since these formulae are not valid in such cases). Examples are given where $\tau 0$, $\tau 1$, C0, C1, and three values of L* are computed for cells with different geometries and different degrees of soma shunt. When a cell is best represented by two cylinders of unequal length, L* computed using $\tau 0/\tau 1$ may be greater than or equal to the sum of the actual L values of the two cylinders (see also Segev and Rall, 1983). Taper tends to increase $\tau 0/\tau 1$ and C0/C1 giving an underestimate of the true L. When a large shunt exists at the soma, C0 is very small making L* computed using C0/V(0) or C0/C1 very large (with little regard for cell geometry). In this case $\tau 0$ may be a very poor estimate of the time constant of dendritic membrane.

Although one can derive formulae for L for structures other than cylinders, the complication of non-uniform Rm limits their usefulness. Instead, we solve the constrained inverse problem: given experimental parameter estimates of $\tau 0$, $\tau 1$, C0, C1, input resistance, soma membrane area, and an approximate simplified geometry, find (using suitable constraints) values for the soma and dendritic time constants, the L of each segment, and the dendritic to soma membrane area ratio. This has been done for cells represented as cylinders with a soma shunt using Newton's method. Resolution of model parameter values depends on the constraints chosen and on the accuracy of experimental parameter estimates.

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423.1 ONTOGENY OF CYTOSOLIC ANDROGEN RECEPTORS IN THE BRAIN OF THE FETAL RHESUS MONKEY. Robert J. Handa, John A. Resko, (SPON: T.P. Condon). Dept. of Physiol., Ore. Health Sci. Univ., Portland, OR 97201 and Div. of Reprod. Biol. & Behav., OR 97006.

Sexual differentiation of the monkey brain occurs during the first trimester of a 166 day gestational period and is androgen dependent. In order to determine if androgen receptors are present in brain tissues during the critical period for sexual differentiation, we measured cytosolic androgen receptor (CAR) in brains of fetal rhesus monkeys at 40-50, 65, 80, and 150 days of gestation. We performed saturation analysis of CAR on tissues from 150 day fetuses using ^{3}H-R1881 as the ligand. Pools of cytosols derived from hypothalamus, preoptic area, amygdala and septum (HPAS) revealed a single, saturable, high affinity binding site with an apparent K_d of 1.5 X 10^{-10}M which was similar to adult male rhesus HPAS (0.8 X 10^{-10}M). ^{3}H-R1881 binding was specific for androgen and was removed by competition with radioinert testosterone (T) and dihydrotestosterone, but not with progesterone or 17β-estradiol.

Single point estimates of CAR were made in hypothalamus-preoptic area (HPOA), frontal cortex (FC) and temporal cortex. In the HPOA there was a significant age-dependent increase in CAR from 40-50 to 150 days of gestation (2.1 ± 0.5 fmol/mg protein, n=4♂ and 3♀ ; 6.2 ± 0.3 fmol/mg protein, n=4♀ , respectively; $p < 0.5$). There were no sex differences. In contrast, CAR numbers in FC were highest at 40-50 days of gestation. In addition, there was an apparent sex difference in the 40-50 day fetuses (5.5 ± 1.0 fmol/mg protein in five males compared to 1.9 fmol/mg protein in two females). CAR levels in FC declined to 0.7 ± 0.1 fmol/mg protein by 150 days of gestation (n=4♀ 's).

Treatment of pregnant females with T propionate (25 mg/day, from days 40 to 50) resulted in elevated serum T levels in females at 50 days (6.0 ± 2.0 vs 0.22 ± 0.5 ng/ml in untreated controls), whereas T levels in male fetuses were not different from untreated controls (4.4 ± 1.7 ng/ml, n=4, vs 3.7 ± 0.5 ng/ml, n=5). CAR levels in FC were elevated in 50 day T-treated female fetuses (4.4 ± 1.3 pmol/mg protein, n=3) but not in 50 day T-treated males (5.1 ± 0.7 fmol/mg protein, n=4) compared to untreated controls. CAR in the HPOA did not differ following T treatment.

These data provide new information regarding the ontogeny of CAR in brain tissue of fetal rhesus monkeys. We observed transitory elevations of CAR in cortical tissue in early gestation which seem to be controlled by T. This may be an important observation for understanding the cellular components of sexual differentiation of the primate brain.
(Supported by NIH 16022)

423.2 AUTORADIOGRAPHIC STUDIES OF ECDYSTEROID BINDING IN THE NERVOUS SYSTEM OF MANDUCA SEXTA. S.E. Fahrbach & J.W. Truman. Dept. of Zoology, University of Washington, Seattle, WA 98195.

We are attempting to develop autoradiographic techniques to identify neurons capable of binding radiolabeled ecdysteroids in the nervous system of the moth Manduca sexta. Individual abdominal ganglia are dissected and incubated in 3H-ponasterone A, a biologically active phytoecdysone previously used to characterize ecdysteroid receptors in Drosophila imaginal disks and the Kc cell line. The tissue is rapidly frozen. Cryostat sections are cut under safelight conditions and thaw-mounted directly onto cleaned glass slides previously coated with Kodak NTB-3 nuclear track emulsion. Autoradiograms are developed, fixed, stained, and examined after exposure times of 3 to 6 months. Tissue was taken from moths that had been ligated at the thoracic-abdominal junction in order to eliminate endogenous ecdysteroids, which are produced by the prothoracic glands of the thorax. Experimental parameters that were systematically varied included concentration of ligand, length of incubation, number of post-incubation rinses, method of freezing, section thickness, and exposure duration. Control moths were either pretreated with an excess of unlabeled 20-OH-ecdysone (the native hormone) before incubation in the tritiated ligand, or were processed without the ligand.

Evidence of nuclear concentration of 3H-ponasterone A was obtained in discrete populations of neurons in abdominal ganglia of both larval (5th instar) and adult Manduca sexta. Examples of both motoneurons and interneurons were found that exhibited apparent binding of the steroid hormone. No labeled cells were present in tissue from moths pretreated with unlabeled 20-OH-ecdysone, and no evidence of chemography was found in autoradiograms produced from tissue not treated with the tritiated ligand.

Ecdysteroids are powerful regulators of many aspects of development of the moth nervous system, including neuronal morphology, responses to peptide hormones, differentiation of post-embryonically-generated neurons, and cell death. Mapping ecdysteroid target cells in the developing nervous system of Manduca sexta will guide future studies of the mechanisms underlying the actions of these steroid hormones on specific, identified neurons.

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423.3 ESTROGEN RECEPTORS IN THE BRAINS OF NEONATAL GRAY SHORT-TAILED OPOSSUMS (MONODELPHIS DOMESTICA). B.H. Fadem* and A.M. Etgen (SPON. H. Edinger). Dept. Psychiat. U.M.D.N.J. - N.J. Med. Sch., Newark, N.J. 07103 and Depts. Psychiat. and Neurosci., Albert Einstein Coll. Med., Bronx, N.Y. 10461.

Marsupials are useful models for studying the role of estradiol in the organization of mammalian reproductive behavior since they are readily accessible at a sexually immature stage of development and do not possess systems to protect them from maternal or exogenously applied estrogens. Recently, treatment with estradiol benzoate on days 1 and 3 of postnatal life was shown to block testicular development and to result in physical and behavioral feminization of genetically male gray opossums (Fadem and Tesoriero, 1986, Biol. Reprod. 34:771). Since the presence of estrogen receptors may be related to critical periods for estrogen action on the developing brain, the present study was designed to determine when neural estrogen binding sites appear during neonatal development in gray opossums.

The subjects of this study were neonatal gray opossums sacrificed on postnatal day 1, 4, 8 or 16. To conduct the study, whole brains of animals of each age were pooled, homogenized and a cytosol fraction prepared by centrifugation. Blood samples were also collected in heparinized tubes at the time of sacrifice. Receptor levels were assayed in the cytosol fraction by incubating aliquots with radiolabeled estradiol in the presence or absence of excess unlabeled estradiol. Levels of serum estradiol were assayed by radioimmunoassay procedures by Dr. Mary Erskine, Boston University.

Estrogen binding sites were undetectable in cytosols from one-day-old gray opossums. However, both 4-day-old and 8-day-old gray opossums had measurable levels of 3H-estradiol binding in whole brain cytosols. The binding was of high affinity in both groups (Kd=0.23 nM in 4-day-old and 0.31 nM in 8-day-old pups), and the number of binding sites increased from 2.99 to 4.02 fmole/mg protein between days 4 and 8. Brain cytosols from 16 day-old gray opossums also showed significant binding, and sufficient cytosol was available to demonstrate that 3H-estradiol was displaceable by diethylstilbestrol and hydroxytamoxifen but not by progesterone or dihydrotestosterone. Levels of circulating estradiol were high in 4- and 8-day-old gray opossums (324 and 301 pg/ml respectively). Findings of both estrogen receptors and of the presence of high levels of circulating estrogen during early postnatal life, suggest that estrogen may play an important role in development in this marsupial species.

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423.4 SELECTIVE ANDROGEN EFFECTS ON RAT SPINAL MOTONEURON MORPHOLOGY AND CONNECTIVITY. E.M. Kurz, C.A. Bowers, and D.R. Sengelaub. Dept. of Psychology, Indiana University, Bloomington, IN 47405.

In male rats, the spinal nucleus of the bulbocavernosus (SNB) projects to the perineal muscles bulbocavernosus (BC) and levator ani (LA), and the dorsolateral nucleus (DLN) projects to the ischiocavernosus (IC) muscle. Adult females normally lack these muscles; however, females treated prenatally with the androgen dihydrotestosterone propionate (DHTP) retain the SNB target muscles, which are now anomalously innervated by motoneurons in the DLN (Breedlove '85). In normal males the morphology of DLN and SNB motoneurons differs significantly (Kurz, Sengelaub, and Arnold, '86); to investigate the factors which determine neuronal morphology and connectivity, we compared SNB and DLN motoneurons having either normal or hormonally altered specificity.

Timed pregnant rats (Sprague-Dawley) received DHTP (2 mg/day in oil, SC) from embryonic (E) days 17 to 22; at birth (E23) pups were cross-fostered to other lactating females. At 60 days of age DHTP-females were given testosterone implants to increase muscle mass for reliable identification. Motoneurons were visualized by injecting 0.5 ul of cholera toxin-HRP (0.2%) unilaterally into either the BC, LA, or IC muscles of DHTP-females or normal males at 83-85 days of age (n= 4-6 per group). After 48 hours cords were processed with TMB and the number, location, soma size, number and orientation of primary processes, and distribution and amount of dendritic arbor of HRP-labeled cells were recorded.

In both groups virtually all labeled motoneurons were located in the DLN after IC injection or the SNB after LA injection. After BC injection, 97% of labeled cells were in the SNB of males but only 47% were in the SNB of DHTP-females; the remaining 53% were located in the DLN. In males and DHTP-females the morphology of SNB motoneurons projecting to the LA (SNB-LA) was identical ($\bar{x}$ soma area= 1148 um², $\bar{x}$ number of primary processes =7.1, similar process orientation, $\bar{x}$ arbor per cell= 6360 um). SNB-BC and SNB-LA motoneurons were identical in males, but SNB-BC motoneurons in DHTP-females had smaller somas (890 um²) and less arbor per cell (1670 um). DLN-IC motoneurons in DHTP-females were smaller than those of males in soma size (1050 vs. 750 um²), number of processes (5.4 vs. 4.9), and arbor per cell (2410 vs. 1720 um). DLN-IC and DLN-BC motoneurons in DHTP-females were identical, but compared to SNB-BC motoneurons the DLN-BC motoneurons had smaller somas (682 um²) and fewer processes (4.6), oriented differently.

Motoneuron morphology is thus not determined by peripheral targets and only partially affected by the local microenvironment of the spinal cord. Moreover, prenatal DHTP treatment in females selectively influences motoneuron morphology and alters the specificity of motoneurons projecting to only one of the SNB's normal targets. (Supported by NIH grant NS24877)

423.5 SEX DIFFERENCE IN DENDRITIC DIMENSIONS OF NEURONS IN THE DORSAL POA/AH OF THE FERRET. J.A. Cherry*, S.A. Tobet, M.J. Baum, and T. J. DeVoogd, Dept. Biology, Boston University, Boston, MA 02215 and Dept. Psychology, Cornell University, Ithaca, NY 14853

A sexually dimorphic nucleus has recently been identified at the dorsal border of the preoptic and anterior hypothalamic areas (POA/AH) in the male ferret (Tobet et al., Brain Res. 364:249, 1986). Somal areas of neurons in the male nucleus (MN-POA/AH) are larger than areas of cells in a corresponding region of the female POA/AH. To determine whether this sex difference in somal size and grouping extends to the dendritic structure of individual neurons, POA/AH tissue from adult male (n=5) and female (n=5) ferrets was processed using a Golgi-Cox procedure, sectioned at 120 μm, and counterstained with thionin. The animals were gonadectomized and had received no steroids for 23 (5 males, 3 females) or 5 days (2 females) prior to sacrifice. Multipolar neurons with cell bodies in the MN-POA/AH or in the corresponding POA/AH region of females were studied. A semi-automated microscope and computer system was used to trace dendritic branching of 19 male and 17 female neurons in three dimensions, and somal areas of these neurons were estimated from camera lucida tracings.

Dendritic density (determined by the number of intersections of dendrites with concentric spheres drawn around the cell body) and total dendritic length were both significantly greater for male neurons (mean ± SE; density: 93 ± 7 vs 65 ± 6 intersections, $t(34)=2.85$, $p<0.01$; length: 721 ± 46 vs 576 ± 57 μm, $t(34)=1.98$, $p<0.055$). In contrast, two measures of dispersion, mean distance from the cell body to dendrite tips (males: 102 ± 4, females: 98 ± 7 μm) and median radial distance (the distance from the cell body at which there are equal numbers of intersections proximally and distally; males: 65 ± 3, females: 60 ± 4 μm), were equivalent in males and females. Also, somal areas of the selected multipolar neurons were similar in males (143 ± 7 μm^2) and females (145 ± 9 μm^2.)

Thus, the extension of dendrites from the cell body in the dorsal POA/AH is similar in males and females, whereas dendritic length and density are greater in male neurons. These results suggest that the potential for synaptic contacts within a given radius from the cell body is greater in the POA/AH of males. It is notable that sex differences in dendritic morphology were found in neurons with equivalent somal dimensions. Future research will reveal whether steroidal environment influences sexually dimorphic features of dendritic structure in either multipolar or other types of POA/AH neurons.

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423.6 EARLY ANDROGEN & ESTROGEN DIFFERENTIALLY AFFECT ADULT MORPHOLOGY OF SEXUALLY DIMORPHIC SPINAL NUCLEUS. D.S.Hall, K.M.Rasmussen* & S.M.Breedlove. Dept. Psychology, Univ. California, Berkeley CA 94720.

Both estrogens and androgens have been shown to exert masculinizing influences upon CNS morphology and behavior in vertebrates. The motoneurons of the spinal nucleus of the bulbocavernosus (SNB) innervate the bulbocavernosus (BC) muscles in the perineum. Both the BC and its SNB cells are present in newborn female rats but die shortly thereafter. The involution of the SNB system can be prevented with neonatal testosterone or dihydrotestosterone propionate (DHTP) treatment, but not by estradiol benzoate (EB) alone. Because it had not yet been established whether EB, in the presence of androgen could masculinize the SNB system, we treated newborn female rats with EB which did not spare SNB motoneurons from death either alone or in conjunction with DHTP. However, early EB treatment did result in larger SNB cell somata and nuclei in adulthood.

Gonadally intact newborn female rats were injected with either 0.05 ml sesame oil alone, or oil with 100 μg EB, or 1 mg DHTP, or both EB and DHTP. The rats were overdosed with pentobarbital at 59-63 days of age and perfused with saline and formalin. Spinal cords were removed, frozen-sectioned in 50 μm transverse slices and stained with thionin. The number of motoneurons in the SNB region was counted and corrected for split nuclei error. SNB somata and nuclei were drawn with a camera lucida, their cross-sectional area measured with a computer-aided digitizing tablet and analyzed via 2-way ANOVA of the 4 groups (EB treated or not across DHTP treated or not).

	Number SNB cells, SEM (N)		SNB cell soma area (μm^2)	
	- DHTP	+	- DHTP	+
EB -	78.4 ±3.8(6)	150.8 ± 6.6(6)	929.8 ±30.1(11)	997.9 ±46.8(10)
EB +	88.7 ±8.4(6)	140.4 ±15.3(7)	977.6 ±24.2(10)	1137.3 ±32.2(11)

Neonatal DHTP permanently masculinized both the number and the size of SNB motoneurons ($p<.01$). EB had no effect on the number of SNB cells either alone or in conjunction with androgen($p>.9$). Neonatal EB did significantly increase the adult size of SNB somata and nuclei ($p<.01$). There was no significant interaction of EB and DHTP for any measure ($p>.2$), indicating that EB and DHTP exert their influence on SNB size independently. Since in adulthood SNB motoneurons accumulate androgens but not estrogens, and because EB is known to masculinize the some developing brain sites, these two metabolites of testosterone may normally act in concert via different mechanisms (perhaps at different sites) to increase SNB size.

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423.7 NEONATAL CASTRATION PROMOTES MALE PLASTICITY TO DIFFERENTIAL ENVIRONMENTS IN HIPPOCAMPAL DENTATE GRANULE NEURONS. J. R. Kopcik, J. M. Juraska & D. L. Perry* Dept. of Psych. and Neural & Behavioral Biology Program, Univ. of Ill., Champaign, IL 61820

We have previously demonstrated that females exhibit dendritic plasticity in hippocampal dentate granule neurons in response to differential environments, while males do not (Juraska et al. 333:73,1985). Sex differences also were found within each environment such that males had more dendritic material than females when raised in an isolated environment, while females had more dendritic material than males following rearing in a complex environment. In the present experiment we investigated whether testosterone (developmental and post-puberal) was responsible for the male pattern of results.

Male rat pups were castrated or sham operated within 6 hours of birth and returned to their mothers. At weaning, littermate sets of these rats were assigned to either an isolated environment or a relatively complex environment in which rats of each hormonal condition were separately group housed with objects that were changed daily. After a month of differential rearing, the rats were sacrificed; the brains were Golgi-Cox stained and cut into coronal 150 micron sections. Twenty neurons per animal were traced from 7 litters (28 animals), and intersections between dendrites and a series of concentric rings (20μ intervals) were counted.

A concentric ring analysis revealed that hormonal condition and environment significantly interacted in the middle rings: 5, 6, 8, 9 and 10. Post hoc tests indicated that sham operated males did not show any differences in ring intersections, but castrated males from the complex environment did have more dendritic intersections than isolated castrate males. Thus castration appeared to mimic (although to a lesser degree) the female pattern of plasticity. However, the increased dendritic length in females compared to males from the complex environment was not found in the castrated males. Instead, there was a significant effect of hormonal condition in rings 10 through 14 such that sham males had more dendritic material than castrated males regardless of environment. Thus neonatal castration promotes the female pattern of plasticity but not the female pattern of sex differences in the complex environment.

Supported by NSF.

423.8 EXCESS TESTOSTERONE DURING EARLY POSTNATAL DEVELOPMENT DOES NOT CHANGE THE GROSS SIZE OF THE RAT VISUAL CORTEX. J. M. Juraska, J. R. Kopcik, J. H. Y. Kim*, S. N. M. Reid* & E. B. Nussbaum*, Dept. of Psych. and Neural & Behavioral Biology Program, Univ. of Ill., Champaign, Il 61820

Geschwind and Behan (Proc. Natl. Acad. Si. 79:5097, 1982) proposed that testosterone slows, and in extreme disrupts, left hemisphere development which would account for the greater incidence of learning disorders in males. We attempted to test this hypothesis by providing an excess of testosterone to male rats during their early postnatal development and examining the visual cortex, an area that we have demonstrated to be sexually dimorphic (Juraska, Brain Res. 295:27, 1984) and likely to be influenced by neonatal testosterone.

Male rats were injected with 500μg of testosterone proprionate in oil on days 3, 5 and 7. Control littermates of both sexes were injected with oil alone on the same days. At 23 days of age all rats were perfused with paraformaldehyde, their brains were frozen sectioned at 40 microns and stained with cresyl violet. The visual cortex (areas 17 and 18) was examined in rats from four litters (26 animals) at one anterior and one posterior level. Four measurements of cortical depth and individual layer depth were taken at intervals scaled to the size of each brain in both hemispheres.

There were no statistically significant effects of excess testosterone in the cortical depth measures, nor were there hormonal condition by hemisphere interactions. At this point the data do not support the Geschwind and Behan hypothesis. We are currently examining cell density in the visual cortex andextending our measures to other cortical areas.

Supported by NIMH.

423.9 POSTNATAL ONTOGENY OF SEROTONIN AND SUBSTANCE P IN THE SEXUALLY DIMORPHIC CREMASTERIC NUCLEUS. M.A. Romagnano, B.W. Newton & R.W. Hamill. Departments of Neurology and Medicine, University of Rochester/Monroe Community Hospital, Rochester, NY 14603.

The rat cremasteric nucleus lies in the L1-2 ventral spinal gray. In males this nucleus innervates the cremaster m.; in females the peripheral target is unknown. Previous serotonin (5HT) and substance P (SP) immunohistochemical and morphological studies reveal sexual dimorphism of this nucleus with males containing greater or almost exclusive amounts of immunohistochemically demonstrable 5HT and SP, respectively and 57% more cremasteric neurons (Anat. Embryol. 170:117,'84; Brain Res. 301:243,'84; Brain Res. Bull. 15:609,'85 Neurosci. Lett. 64:157,'86).

This study was performed in order to determine the ontogeny of 5HT and SP in the male rat cremasteric nucleus. Rats (4/age group; postnatal day (P) 0, 4, 8, 10, 20, 40, adult (>P60)) were perfused either via a constant rate pump (P0-P10; 2.0ml/min.) or by gravity with modified Zamboni's fixative. Spinal cords were removed and placed in fresh fixative until horizontal sectioning at 70um (P0-P10) or 40um (P20-adult) with a vibratome. The free-floating PAP technique was used to demonstrate the presence of 5HT (1:8000, overnight incubation; Brain Res. 376: 155,'86) and SP (1:8000, overnight incubation; IncStar, #17H2T).

Adult SP is found surrounding clusters of cremasteric motor neurons and their processes which extend either medially through the ventral horn and into the ventral funiculus or laterally into the lateral funiculus. In addition large dense bundles of SP fibers, forming intraspinal pathways, are observed extending dorsally into laminae III and IV. During ontogeny, scant SP is observed at P0 in these dorsal pathways which rapidly accumulate SP fibers and approach adult densities by P8. At P0 cremasteric motor neurons are surrounded by scant numbers of SP fibers which increase in number at P4-P10 and approach adult levels by P20.

Adult 5HT fibers are varicose, tortuous, and surround cremasteric motor neuron clusters. Most cremasteric neuron processes, surrounded by dense 5HT fibers, extend medially into the ventral funiculus with smaller numbers extending dorsally and laterally. Dorsal projecting 5HT < dorsal SP. During ontogeny, P0-P8 5HT fibers are barely discernable in the cremasteric nucleus. At P10 a dramatic increase in 5HT fibers is observed with these fibers approaching adult numbers. At P20-P40 the density and distribution of 5HT fibers in the cremasteric nucleus resembles adults.

These data indicate that 5HT and SP apparently develop independently of each other in the cremasteric nucleus. However, the appearance of each transmitter may be related to postnatal testes descent and the regulation of testes temperature and pain perception in the scrotal region. This research was supported by UofR/MCH research funds and NIH grant NS22247 to MAR.

423.10 5HT AND 5HIAA IN THE SEXUALLY DIMORPHIC CREMASTERIC NUCLEUS: MORPHOLOGICAL, BIOCHEMICAL AND PHYSIOLOGICAL CORRELATIONS. B.W. Newton & R.W. Hamill. Depts. of Neurology and Medicine, Univ. of Rochester/Monroe Community Hosp., Rochester, NY 14603.

The rat cremasteric nucleus lies in the L1-2 ventral spinal gray. In males this nucleus innervates the cremaster m. and may play a role in testes temperature regulation; in females the peripheral target is unknown. Serotonin (5HT) immunohistochemistry (IHC) and morphological studies reveal sexual dimorphism of this nucleus with males containing greater amounts of IHC demonstrable 5HT and 57% more cremasteric neurons than females (Brain Res. Bull. 15:609,'85). The present studies were performed to determine: 1) if the amount of 5HT, and a metabolic product 5-hydroxyindolacetate (5HIAA), in the cremasteric nucleus is sexually dimorphic as compared to IHC evidence; 2) if cremasteric 5HT and 5HIAA levels are dependent upon postnatal hormonal support in males; and 3) if sexually dimorphic differences exist in 5HT and 5HIAA levels in cold stressed rats. Male and female rats (n=7; P50) were killed via CO_2 asphyxiation and the L1-2 spinal levels rapidly removed and frozen on dry ice. The ventral gray horn was micro-punched, placed in 0.1N perchlorate, sonically disrupted, and the supernatant assayed for 5HT and 5HIAA via HPLC with coulometric detection. In addition, littermates (n=7) were castrated within 12h of birth, allowed to mature (P50), then assayed as above. Other male, female, and castrate littermates (n=4/group) were kept at 4°C for 6 or 12hr, then assayed as above. 5HT IHC was performed using the PAP technique (Brain Res. 376:155,'86). Male and female data were expressed three ways. Results expressed per nuclear group indicate the male cremasteric nucleus contains more 5HT and 5HIAA (5320pg±317 ($\bar{x}$ ±S.E.M.) and 1777±106pg) than females (4240±283 and 1570±145pg); when expressed as pg/ug protein the female cremasteric nucleus contains more 5HT and 5HIAA (111±9 and 36.9±5.9pg) than males (71±7 and 23.6±2.0pg). However, if the difference in neuron number, which may reflect protein content of the nucleus, is taken into account and the male values for 5HT and 5HIAA are increased by 57% then females and males contain 111±9 and 110±11pg (corrected value) of 5HT/ug protein and 36.9±5.9 and 36.9±3.1pg (corrected value) of 5HIAA/ug protein, suggesting that the IHC 5HT dimorphism observed (male>female) is a function of the number of neurons being innervated by 5HT vs the male rat receiving more 5HT per neuron than females. Thus, the target cremasteric motor neurons regulate afferent 5HT input. Castration data suggests that 5HT and 5HIAA levels are independent of postnatal testosterone. Cold stress paradigms suggest 5HIAA/5HT ratios, a marker of 5HT neuron activity, increases at 6hr in all groups but by 12hr the ratios become divergent. Supported by UofR/MCH research funds.

423.11 THE SEXUAL DIFFERENTIATION OF THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS IS ANDROGEN DEPENDENT IN THE HOUSE MOUSE. Christine Konen Wagner* and Lynwood G. Clemens. Dept. of Zoology, Michigan State University, East Lansing, MI 48824

The spinal nucleus of the bulbocavernosus (SNB), located at L4 and 5 in the rat, is a nucleus of motorneurons innervating muscles of the perianal area. This nucleus is sexually dimorphic, with females having fewer and smaller cells than males. Treatment of female rats with testosterone propionate (TP) during late gestation significantly increased the number of SNB neurons found in adulthood, (Breedlove & Arnold, 1983). The SNB is sexually dimorphic in the house mouse as well. The purpose of the present study was to determine whether the development of the SNB in the mouse was affected by perinatal androgen treatment.

Hybrid female pups, resulting from a cross between a DBA/2 male and a C57BL/6 female, were treated with TP during one of four developmental periods: early gestation (EG), 200ug TP was given to pregnant dams on days 11, 12, 13, and 14 of gestation; late gestation (LG), 200ug TP on days 14, 15, 16, and 17 of gestation; early postnatal (EP), 100ug TP was administered to pups on days 1, 3, and 5; late postnatal (LP), 100 ug TP on days 7, 9, and 11. Control females received an egual volume of the sesame oil vehicle alone during the same developmental periods. Spinal cords were removed between 60 and 90 days of age. Alternate 60um sections were examined with Nissl stain and SNB cell number, and cross-sectional somal area were determined using image analysis processing.

Mean cell numbers and mean somal areas (S.E.M.) are reported in the following table.

	CELL NUMBER		SOMAL AREA(um)	
	TP	OIL	TP	OIL
EG	32.8 (5.8)*	11.6 (2.2)	521.1 (17.6)*	431.9 (23.8)
LG	32.4 (7.2)*	11.2 (1.0)	441.6 (17.0)	453.5 (30.6)
EP	43.6 (7.5)*	8.0 (1.8)	489.6 (16.7)	473.7 (28.3)
LP	21.6 (4.0)	9.6 (1.7)	485.4 (21.8)	412.5 (30.2)

* -significantly different from oil control

Analysis of variance with post hoc analysis revealed that androgen treatment significantly increased cell number during EG and LG ($p<0.05$) as well as during EP ($p<0.01$). Cell number was not significantly changed by TP treatment during LP. Somal area was significantly increased by androgen treatment during EG.

These results indicate that the sex differentiation of the SNB in the mouse is regulated by androgens both prenatally and postnatally. Androgens had the greatest effect when administered during early postnatal life, (days 1, 3 and 5). This effect was absent when androgens were given later in postnatal life, (days 7, 9 and 11). These results contrast those of the rat in which the effect of androgen was absent when administered after birth. Similar to results with the rat, the sensitive period for the TP effect on cell size differed from the sensitive period for the TP effect on cell number, suggesting that separate mechanisms exist for the two parameters of development. It has been proposed that androgens act on SNB development by preventing normally occuring cell death. Determining the sensitive period for androgen effects is an important step in further understanding the mechanism underlying this process.

423.12 ANDROGEN BLOCKADE OF BULBOCAVERNOSUS MUSCLE INHIBITS TESTOSTERONE-DEPENDENT MASCULINIZATION OF SPINAL MOTONEURONS IN NEWBORN FEMALE RATS. Renata B. Fishman & S.M.Breedlove, Dept. Psychology, Univ. California, Berkeley, CA 94720.

The sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) and its target perineal bulbocavernosus (BC) muscles are present in adult male, but not female, rats. Androgen treatment of newborn females masculinizes both SNB cell number and BC muscles, by sparing SNB motoneurons from programmed cell death and preventing muscle degeneration. It is not yet clear whether androgen spares the SNB system by interacting with SNB motoneurons and/or muscles. Androgen treatment maintains denervated BC muscles of newborn females, suggesting androgen need not interact with SNB cells to spare the muscles (Fishman & Breedlove, '87, Muscle & Nerve, in press). Furthermore, androgen can spare SNB cells which are androgen-insensitive, suggesting that androgen may act to stabilize the muscle and thereby indirectly save the motoneurons (Breedlove, '85, Soc. Neurosci. Abstr.,11, 160.) The present study further examines the role of androgen action on the BC muscles in masculinizing SNB cell number. We studied the effect of systemic androgen on SNB motoneuron number following blockade of androgen receptors in BC muscle by local administration of flutamide in newborn female rats.

Newborn Sprague-Dawley female rat pups were anesthetized by hypothermia and then randomly assigned to receive one of three injection regimens on days 1 and 3 of life. Each injection regimen consisted of four injections, two (bilateral) into the perineal compartment containing the BC, and two s.c., rostral to the interscapular fat pad. All pups received 1 mg testosterone propionate (TP) in sesame oil (.05 ml) s.c. and either: 1) Flutamide (Schering, total bilateral dose= 25 µg in 1 µl propylene glycol (PG) at the muscle, and 1 µl PG vehicle s.c. (N=9); 2) PG at the muscle, & flutamide s.c. (N=8); or 3) PG at the muscle, & PG s.c. (N=5). Animals were sacrificed between days 21 and 26 of life, perfused with 9% saline and buffered formalin, and their spinal cords and perineal muscles were removed. Spinal cord segments between L5-L6 were frozen sectioned at 50 microns, stained with thionin, and the number of SNB cells counted.

The number of SNB cells was slightly but significantly lower in rats receiving flutamide at the muscle than in rats receiving the flutamide s.c. (t-test, $p< .05$). Apparently this dose of flutamide, when injected systemically, is too low to inhibit androgenic masculinization, since there was no significant difference in the number of SNB cells in the flutamide s.c./BC vehicle group and the group receiving vehicle both at the muscle and s.c. Thus, blocking androgen action locally at the muscle will decrease the number of SNB motoneurons spared. This result further supports the idea that the BC muscles are an important site of androgen action for sparing SNB system structures during development.

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423.13 CRITICAL PERIOD FOR ANDROGENIC REGULATION OF SOMA SIZE OF SEXUALLY DIMORPHIC MOTONEURONS IN RAT SPINAL CORD.
J.H. Lee*, C.L. Jordan, and A.P. Arnold (SPON: N.J. Woolf). Dept. Psychology, UCLA, Los Angeles CA 90024.

The spinal nucleus of the bulbocavernosus (SNB) is a sexually dimorphic group of motoneurons in rat spinal cord that innervates perineal muscles. Male SNB motoneurons are more numerous and larger than female SNB motoneurons. These cellular attributes are regulated developmentally by androgen. Androgen treatment of neonatal females permanently masculinizes (increases) the size and number of SNB neurons. The purpose of this study was to determine the end of the critical period for this androgenic regulation of SNB soma size in females.

Female Sprague-Dawley rats each received three injections of 1mg testosterone propionate dissolved in oil during one of four postnatal developmental periods. Controls received oil alone. Animals received injections on postnatal days (d) 1, 3, and 5 (period 1); on d7, 9, and 11 (period 2); on d13, 15, and 17 (period 3); or on d19, 21, and 23 (period 4). At 65 days of age, all animals were gonadectomized and implanted with a Silastic implant (40 mm long, .125 in OD, .062 in ID) containing testosterone. All rats were sacrificed at 95 days of age and their spinal cords were sectioned at 50 um and stained with thionin. The cross-sectional areas of 25-32 SNB somas per animal were measured using a computerized digitizing tablet interfaced with the microscope. All groups were compared with normal males and females that received no neonatal treatment but the same adult treatment as the other groups.

As reported previously (Breedlove and Arnold, 1983), neonatal TP injections masculinize the soma size of female SNB motoneurons if given at postnatal days 1-5 or 7-11 ($p<.0001$, ANOVA). Androgen injections after this period do not have a significant effect ($p>.05$).

	TP	OIL	
period 1 (d1-5)	1047 ± 39 (6)	733 ± 17 (3)	soma area
period 2 (d7-11)	935 ± 14 (6)	768 ± 15 (6)	square um
period 3 (d13-17)	833 ± 11 (6)	766 ± 20 (4)	mean ± SE
period 4 (d19-23)	809 ± 27 (6)	795 ± 21 (3)	(n/group)
normal males:	1132 ± 38 (6)		
normal females:	688 ± 9 (6)		

These data imply that the critical postnatal period for permanently increasing female SNB soma size ends around the second week after birth. This information may help delineate what cellular events underlie the critical period for permanent androgenic regulation of cell size. Supported by NIH grant HD15021.

423.14 ANDROGEN EXPOSURE PERINATALLY INCREASES THE NUMBER OF NEURONS IN THE CENTRAL PART OF THE MEDIAL PREOPTIC NUCLEUS OF THE FEMALE RAT.
R.E. Dodson*, J.E. Shryne* and R.A. Gorski. Dept. of Anatomy, Lab. of Neuroendocrinology, Sch. of Med., Univ. of California, Los Angeles, CA 90024.

The Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA) of the rat is larger in volume in males than in females, is hormone sensitive and in the female can be sex-reversed by exposure perinatally to testosterone propionate (TP). Moreover, the genesis of neurons destined for the SDN-POA is prolonged and a subcomponent of SDN-POA cells can be permanently labeled by exposure to ^{3}H-thymidine on day 18 of gestation (E18). The present experiment was performed to answer three questions: 1) Are cells labeled with ^{3}H-thymidine on E18 evenly distributed throughout the SDN-POA, 2) Are these late arising cells steroid sensitive during development; and 3) Is the effect of steroid exposure on the number of cells labeled with ^{3}H-thymidine on E18, related to neurogenesis? Pregnant Sprague Dawley rats were injected with ^{3}H-thymidine (5 µCi/g b.w.) on E18 through the jugular vein under ether anesthesia. Daily injections of TP (2 mg/day) were given to mothers starting on either E16 or E20 through the day of birth, and then to pups (100 µg/day, s.c.) through postnatal day 10. At 30 days of age, the rats were perfused transcardially and their brains processed for paraffin sectioning and autoradiography. Preliminary evaluation of the autoradiograms showed that the distribution of labeled cells was restricted to the posterior SDN-POA but could be more accurately described in terms of the cytoarchitectonic subcomponents of the Medial Preoptic Nucleus (MPN) rather than in terms of the SDN-POA. The label was localized to the central division (MPNc). Approximately 18% of the neurons in the MPNc were labeled with ^{3}H-thymidine whereas only 3% of the neurons in an area immediately surrounding the MPNc and 4% in a region anterior to the MPNc were labeled. The volume of the MPNc of females (1.55 ± 0.24 mm^{3}x10^{-3}) was about one third that of males (4.69 ± 0.23). This same pattern was also seen in the total number of neurons (202 ± 9 in females vs. 633 ± 66 in males) and the number of labeled cells (38 ± 5 in females vs. 109 ± 9 in males). All three parameters were increased to those of males in females treated with TP started on E16 (5.27 ± 0.56 mm^{3}x10^{-3}; 779 ± 61 neurons; 137 ± 14 labeled cells) or on E20 (5.61 ± 0.23 mm^{3}x10^{-3}; 682 ± 58 neurons; 131 ± 20 labeled cells). In summary, cells labeled with ^{3}H-thymidine on E18 are localized to the MPNc. Females can be completely sex-reversed in terms of MPNc volume, its total number of neurons and number of E18 labeled cells by exposure to TP perinatally. Starting TP injections on E20 (after neurogenesis is virtually complete) is as effective as starting them on E16 (while neurogenesis is still in progress). This observation suggests that steroid effects on MPNc neurons are not related to neurogenesis. (Supported by HD-01182 & HD-07228.)

423.15 DURATION OF THE ANDROGEN SENSITIVE POSTNATAL PERIOD FOR DIFFERENTIATION OF THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA IN MALE AND FEMALE RATS.
R. W. Rhees*, J. E. Shryne* and R. A. Gorski. (SPON: D. E. Fleming). Department of Anatomy and Laboratory of Neuroendocrinology, UCLA School of Medicine, Los Angeles, CA 90024.

This laboratory has demonstrated the existence of a marked sex difference in the volume of an intensely staining component of the preoptic area of the rat brain. The volume of this component, which is called the sexually dimorphic nucleus of the preoptic area (SDN-POA), is several fold larger in adult males than in adult females. The volume of the SDN-POA can be influenced significantly by the hormone milieu during early postnatal life. Castration of 1-day old male pups reduces the volume of the SDN-POA in the adult. Injection of testosterone propionate (TP) into day 1 castrated pups on postnatal day 2 restores SDN-POA volume to the male level. In addition, injecting female pups on postnatal day 2 or 4 with TP increases SDN-POA volume in the adult. The purpose of the present study was to determine the duration of the sensitive postnatal period during which exogenous androgen administration significantly influences SDN-POA volume in castrated males or intact females. Male pups were castrated under cold anesthesia on the day of birth (day 1). On postnatal days 2, 3, 4, 5, 6, 7, or 8, male and female pups were injected sc with 500 ug TP. Each group consisted of males or females derived from at least 3 different litters. All animals were sacrificed at 30 days of age, their brains serially sectioned at 60 um and stained with thionin. Analysis of the SDN-POA in castrated males showed that TP treatment on days 2, 3, 4, or 5, significantly increased its volume (200 to 300%) over values from oil treated males. In contrast, TP treatment on days 6, 7, or 8, failed to increase SDN-POA volume. A similar pattern was observed in females treated with TP. Females treated with TP on days 2, 3, 4, or 5, showed a significant increase (70 to 130%) in the SDN-POA volume compared to values from oil injected animals, while the same TP treatment in females on days 6, 7, or 8, resulted in no such enhancement. The relative change in SDN-POA volume following postnatal androgen treatment is greater in males than in females. We conclude that (1) SDN-POA development is sensitive to androgen action up through postnatal day 5 and then abruptly becomes androgen-insensitive to this dosage, and (2) although the temporal pattern of response is similar in males and females, androgen exposure postnatally results in greater relative increase in the male SDN-POA volume than in the female's. This greater response may be due to exposure prenatally to endogenous androgen in males. (Supported by NIH Grant HD-01182)

423.16 SEXUAL BEHAVIOR AND MUSCLE DEVELOPMENT OF RATS ALTERED WITH CRITICAL PERIOD ADMINISTRATION OF NICOTINE AND ACTH.
K.J.Rose* A.Segarra*, and F.L.Strand. Biology Dept. New York University. Washington Square, New York, N.Y. 10003.

Our previous studies have shown that the administration of either ACTH fragments or nicotine alters both the maturation of the extensor digitorum longus (EDL)muscle-peroneal nerve complex and the development of motor behavior. This study was designed to identify a critical period for the effects of either of these two molecules on muscle maturation or later sexual performance.

Selective prenatal treatment of pups via the pregnant Sprague-Dawley dams with nicotine tartrate salt(0.25 mg/kg/2X day, i.p.) or ACTH 4-10(10 ug/kg/2X day, i.p.) profoundly affected EDL muscle maturation when viewed in pups two weeks old(postnatal). Contractile parameters of in situ tested EDLs indicate that ACTH 4-10 administration during gestational--GD--days 3 through 12 accelerates muscle maturation; ACTH 4-10 administration during GD days 13 through 21 decelerates muscle maturation via its interaction with the peroneal nerve. Pups treated prenatally with nicotine during either of three gestational periods--G3-8; G9-13; or G14-21--showed accelerated muscle maturation. But, G3-8 treated animals had faster tetanus rise times; G14-21 treated pups had greater tetanus contraction amplitudes.

Pregnant dams were injected on GD 3-21, with either ACTH 1-24 ---10 ug/kg/2x day, i.p.---or nicotine---0.25 mg/kg/2x day, i.p.-- Their male progenies were tested for sexual behavior with a receptive female when 65 days old. Prenatal nicotine treatment adversely affected the sexual behavior of these adult males; 29% showed no sexual behavior. Those that were sexually active had shorter mount latencies, more mounts to the first ejaculation, and fewer ejaculatory series vs controls --$p<0.05$. Like nicotine, ACTH 1-24 also reduced mount latency and mounts to ejaculation, though these differences were non-significant.

These sexual deficits may have been caused by the decreased testosterone levels produced by prenatal nicotine and ACTH during late gestation when neuronal circuits for sexual behavior are being organized.

Thus, our findings indicate that either nicotine or ACTH manipulates the prenatal environment, profoundly affecting both early muscle maturation and later sexual performance.

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424.1 MOLECULAR CHARACTERISTICS OF SOMATOSTATIN RECEPTORS IN PITUITARY CELLS. Kyriaki Thermos and Terry Reisine, Department of Pharmacology, University of Pennsylvania, School of Medicine, Philadelphia, PA.

Somatostatin (SRIF) is a hypothalamic peptide which inhibits the release of a variety of hormones from the anterior pituitary. To study the molecular mechanism by which SRIF regulates pituitary activity, tumor cell lines such as AtT-20, which secretes ACTH, and GH3 which produces growth hormone and prolactin have been highly useful. Functional studies (inhibition of hormone release, cAMP formation and calcium influx) show that SRIF responses in these two cell lines are different. Such functional differences may indicate that the SRIF receptors in the two cell lines are subtypes. SRIF receptor subtypes have been proposed to exist in brain, pituitary, pancreas as well as other tissues. To ascertain whether these functional differences are the result of structural variations in the SRIF receptor we have determined the physical properties of the SRIF receptor in GH3 and AtT-20 cells. SRIF receptors were photocrosslinked to [125I]CGP-23996, a high affinity peptidase resistant analogue of SRIF. Proteins were analyzed by SDS polyacrylamide gel electrophoresis (SDS-PAGE). A single protein was labeled by [125I]CGP-23996 in both AtT-20 and GH3 cells with an apparent molecular mass of 55±5 kD. SRIF, SRIF-28, Trp8-SRIF and Tyr11-SRIF all blocked the binding of [125I]CGP-23996 to the SRIF receptor. Analysis of the [125I]CGP-23996 labeled proteins by two-dimensional PAGE also revealed a single receptor in both cell lines with a pI of 6-6.5. Mixing of the labeled membranes from GH3 and AtT-20 cells followed by two-dimensional PAGE revealed comigration of the SRIF receptors. Proteolytic digestion of the SRIF receptors in GH3 and AtT-20 cells by thermolysin and S. Aureus revealed identical peptide maps supporting the notion that the receptor proteins in the two tissues are very similar. In contrast, pretreatment for 5 min to 12 hr with Trp8-SRIF abolished the labeling of the SRIF receptor in AtT-20 but not GH3 cells. Thus, SRIF receptors in GH3 and AtT-20 cells are functionally distinct but appear to have the same primary structure. Variations in posttranslational processing may therefore be the major means to confer the functional differences. Such processing may be important for creating SRIF receptor subtypes in normal tissue, since preliminary studies show that [125I]CGP-23996 appears to label a protein with the same physical characteristics in brain, pituitary and pancreas. Supported by NIH grants GM34782 and DK37407, and a Grant-in-Aid from the AHA.

424.2 MOLECULAR MECHANISMS OF SOMATOSTATIN INHIBITION OF cAMP-DEPENDENT AND cAMP-INDEPENDENT CALCIUM INFLUX IN THE CLONAL PITUITARY TUMOR CELL LINES, AtT-20 AND GH3. Hung-Li Wang* and Terry Reisine, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia PA.

Somatostatin (SRIF) is a potent and effective inhibitor of hormone release from the anterior pituitary. One mechanism through which SRIF blocks hormone release is by inhibiting adenylate cyclase activity. Another mechanism is to reduce voltage-activated calcium influx. These two responses to SRIF are independently controlled. These findings raise the question whether the same population of SRIF receptors are coupled to adenylate cyclase and calcium channels. To investigate this question, the characteristics of SRIF inhibition of calcium influx stimulated by corticotropin releasing factor (CRF), an activator of adenylate cyclase and K+, a membrane depolarizing agent, in AtT-20 cells, a clonal cell line of the pituitary, were assessed. Changes in cytosolic calcium levels were measured using the fluorescence probe Quin 2. Both CRF and K+ raise cytosolic calcium levels by stimulating calcium influx. SRIF induces an immediate inhibition of CRF and K+ stimulated calcium influx. This effect is concentration dependent and SRIF analogues such as somatostatin 28, Trp8-SRIF and Tyr11-SRIF have the same rank order of potency to block CRF and K+ induced calcium influx. The inhibitory effects of SRIF on AtT-20 cells are abolished by pertussis toxin pretreatment. SRIF inhibition of both CRF and K+ induced calcium influx desensitizes. The desensitization is rapid (T1/2 ~2.5 min) and not due to the degradation of the peptide. Cross-desensitization of somatostatin 28 with SRIF is observed for both the inhibition of CRF and K+ induced calcium influx. These results suggest that a single population of high affinity SRIF receptors, linked to GTP binding proteins, mediate both SRIF's inhibition of cAMP dependent and cAMP- independent regulation of calcium influx in AtT-20 cells. SRIF receptors are also present in another clonal cell line of the pituitary, GH3. The ability of activators of adenylate cyclase, such as forskolin, to increase cytosolic calcium levels in these cells is also blocked by SRIF. The rank order of potency of SRIF analogues to inhibit forskolin stimulated calcium influx is different in GH3 cells than their inhibition of CRF stimulated calcium influx in AtT-20 cells. SRIF inhibition of forskolin stimulated calcium influx in GH3 cells is not transitory as in the AtT-20 cells. These results are consistent with the hypothesis that SRIF receptors in somatotrophs (GH3) and corticotrophs (AtT-20) may be functionally distinct subtypes. Supported by NIH grants DK37407 and GM34782 and a Grant-in-Aid from the AHA.

424.3 **MOLECULAR MECHANISMS OF SOMATOSTATIN DESENSITIZATION** **Nicole Mahy*, Marilyn Woolkalis*, Kenneth Carlson*, David Manning* and Terry Reisine (spon G. Koelle) Dept. Pharmacology, University of Pennsylvania School of Medicine, Philadelphia PA, 19104**

Somatostatin (SRIF) inhibits the release of adrenocorticotropin from the anterior pituitary cell line AtT-20. This inhibition is transitory due to desensitization of SRIF receptors. SRIF desensitization appears to result from an inability of the SRIF receptor to couple to adenylyl cyclase. Pretreatment of AtT-20 cells with Trp8-SRIF reduces the specific binding of [125]I-CGP-23996, a non-reducible SRIF agonist, to the the SRIF receptor. The loss of binding is dependent on the time (T1/2=1-5 min) of pretreatment and is reversible (T1/2=4hrs). The ability of GTP and GTP analogues to inhibit [125]I-CGP 23996 binding is also lost following Trp8-SRIF treatment. SRIF and GTP inhibition of forskolin stimulated adenylyl cyclase activity in AtT-20 cell membranes is also reduced following agonist exposure. GTP inhibition is rapidly lost (T1/2<30 min) and is slow to recover (T1/2>20 hrs). The attenuation of SRIFs inhibition of forskolin stimulated adenylyl cyclase activity takes longer (T1/2>30 min) to occur but is more rapidly reversible (T1/2=30 min). Basal, Gpp(NH)p, GTPgS and forskolin stimulated adenylyl cyclase activity was not affected by the pretreatment. These findings suggest that the stimulatory GTP binding protein(s) are not modified by SRIF pretreatment. This is further supported by the ability of cholera toxin to induce the same level of ADP-ribosylation of Gs in control and SRIF treated membranes. Dideoxyadenosine blockade of forskolin- stimulated adenylyl cyclase activity was the same in control and SRIF-treated membranes suggesting that the catalytic subunit of adenylate cyclase can still be inhibited in the desensitized membranes. The SRIF desensitization involves a reduction in SRIFs stimulation of GTPase activity. Basal GTPase activity, however, is not significantly affected by the SRIF pretreatment. The apparent physical characteristics of Gi/Go, as assessed by 2-dimensional PAGE of the membrane proteins followed by immunoblot analysis using antisera against a region common to G proteins, and the endogenous level of ADP-ribosylation of the G proteins was not clearly modified by the Trp8-SRIF pretreatment. These results, as well as preliminary reconstitution experiments, suggest that inhibitory G proteins are perhaps only subtly modified during SRIF desensitization. The ability of the SRIF receptor to couple to the G proteins is greatly diminished during SRIF desensitization. Studies are in progress to determine what chemical alteration of the SRIF receptor and the G proteins may be responsible for SRIF desensitization. Supported by grants from the NIH GM34782 and DK37407, a Grant-in-Aid from the AHA and funding from the Joint US-Spain Research Committee Fellowship (IPA-8510035).

424.4 EFFECTS OF SOMATOSTATIN ANALOGUES ON DEPHOSPHORYLATION OF THE PRESYNAPTIC PROTEIN, B-50. M.R. Pisano*, D.H. Coy* and L.A. Dokas, Departments of Biochemistry and Neurology, Medical College of Ohio, C.S. 10008, Toledo, Ohio 43699 and Department of Medicine, Tulane University, New Orleans, LA 70112.

Two types of somatostatin receptors exist. One is of high affinity and represented by the single class of receptor found in the pituitary. The second type is unique to brain and of low affinity. The two receptor types can be distinguished by making use of the following somatostatin analogues that differentially bind to the different receptors:

Ala-Gly-Cys-Lys-Asn-Phe-Phe-(D)Trp-Lys-Thr-Phe-Thr-Ser-Cys
[D-Trp^8]-somatostatin

(D)Phe-Cys-Phe-(D)Trp-Lys-Thr-Cys-Thr(OL)
SMS 201-995

(D)Phe-Cys-Tyr-(D)Trp-Lys-Val-Cys-Thr-amide
Peptide III

(D)Nal-Cys-Tyr-(D)Trp-Lys-Val-Cys-Thr-amide
[Nal]-somatostatin

[D-Trp^8]-somatostatin interacts with both receptor types. SMS 201-995 interacts preferentially with the high affinity receptor. Peptide III and [Nal]-somatostatin bind to the pituitary type receptor with high affinity but do not bind to low affinity brain sites. Somatostatin has been shown to alter the phosphorylated state of the presynaptic membrane protein B-50, with characteristics that suggest a receptor-mediated effect on B-50 phosphatase. Using a cortical synaptic plasma membrane assay system, the effects of these analogues from 10^{-8} to 10^{-4} M on dephosphorylation of B-50 have been studied. B-50 is labeled from [γ^{32}P]-ATP for 30" followed by a 30' dephosphorylation in the presence of each peptide. [D-Trp^8]-somatostatin inhibits dephosphorylation of B-50, but only at 10^{-4} M. Peptide III, SMS 201-995 and [Nal]-somatostatin do not inhibit dephosphorylation at any concentration. Similar results are also seen with a semi-purified B-50 phosphatase fraction (ASP 0-55) prepared by extraction of synaptic plasma membranes with Triton/KCl, DEAE-cellulose chromatography and ammonium sulfate fractionation. Studies with these somatostatin analogues indicate that inhibitory effects on B-50 dephosphorylation may correlate with binding of somatostatin to the low affinity receptor. Supported by a grant from the Van Ness-Thompson Foundation and BRS grant 2-S07-RR-057 00-16. Sandoz Pharmaceuticals Corporation is thanked for the donation of SMS 201-995.

424.5 IDENTIFICATION OF A SOMATOSTATIN RECEPTOR IN A MURINE NEUROBLASTOMA CELL LINE (N1E-115). R.W. Barrett* and M. McKinney., Neuroscience Division, Abbott Laboratories, Abbott Park, IL 60064.

Radioligand binding and functional assays were employed to characterize a somatostatin (SS) receptor in the murine neuroblastoma clone N1E-115. [^{125}I-TYR^{11}]SS-14 bound specifically to a single class of sites in membranes prepared from N1E-115 cells (Kd=83±30 pM; Bmax=21,000±2000 receptors/cell). Competitive binding studies with cyclo(N-Me-ALA-TYR-D-TRP-LYS-VAL-PHE) [L363586] did not indicate the presence of putative SS receptors subtypes found in rat brain membranes. In intact cells, SS-14 and its analogs decreased by 30-50% the levels of cyclic AMP induced by forskolin, prostaglandin E1 (PGE1) or vasoactive intestinal polypeptide. The EC50 values for SS-14, SS-28 and L363586 were subnanomolar and comparable to their respective binding constants obtained by competitive binding studies. The effect of SS-14 on cyclic AMP levels was not affected by muscarinic, histamine H1 or opioid receptor blockade. SS-14 did not affect the cGMP levels or release inositol phosphates. Binding of [^{125}I-TYR^{11}]SS-14 to cell membranes was reduced by guanine nucleotides. Pertussis toxin treatment of cells substantially reduced [^{125}I-TYR^{11}]SS-14 binding and the ability of SS-14 to reduce PGE1-stimulated cAMP levels. These data suggest that SS receptors in N1E-115 cells are linked to adenylate cyclase through a G_i protein. The SS receptor system in N1E-115 cells may serve as a model for one of the putative subtypes of SS receptors found in brain.

424.6 ARTIFACTS IN THE MEASUREMENT OF SOMATOSTATIN BINDING SITES IN CORTICAL TISSUE: THE EFFECTS OF FREEZING. D.L. Knight*, C.D. Kilts*, G. Bissette, and C.B. Nemeroff (SPON: E. Busse). Dept. of Psychiatry and Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Decreased regional brain concentrations of somatostatin (SRIF), a tetradecapeptide, have been demonstrated in Alzheimer's disease by several research groups including our own (Soc. Neurosci. Abstr. 9: 1052, 1983). In contrast to findings with other neuropeptide systems, e.g. corticotropin-releasing factor (CRF), SRIF receptors have been reported to decrease in number in the CNS of patients with Alzheimer's disease, in spite of the decrease in SRIF concentration itself (Beal et al., Science 229: 289-291, 1985). We have now scrutinized the SRIF binding assays to determine whether a decrease in SRIF availability results in the expected upregulation of the number and/or function of SRIF receptors, or whether the presently reported results are artifactual. We initiated these studies with a systematic examination of the variables of the receptor binding assay: method of membrane preparation, addition of protease inhibitors and other components to the incubation medium, centrifugation versus filtration as a technique for separating free versus membrane bound radioactivity, and the use of non-frozen versus frozen tissue.

In determining the effects of freeze-thaw cycles on the SRIF receptor we used frontal cerebral cortex of rats as a model receptor system. One half was immediately frozen and the other half was homogenized and used in a binding site saturation analysis using [^{125}I]-Tyr^{11}-SRIF to determine binding site kinetics. We further reserved a portion of the fresh tissue homogenate and froze it at -70°C for later analysis. The frozen portion of the rat cortex and the frozen homogenate were then examined after 24 to 48 hours of storage at -70°C. We observed a highly significant 40% reduction in the number of binding sites (Bmax) in the frozen tissue and greater than 50% reduction in the Bmax of the frozen homogenate. The apparent affinity constant (Kd) was not affected by freezing and thawing. Thus previous results obtained in frozen tissue must now be regarded as problematic.

Human cortical tissue obtained by rapid autopsy (within 30 to 60 minutes after death) is now being used to examine whether compensatory increases in SRIF receptor number occur in Alzheimer's disease. (Supported by MH-40524, MH-39415, MH-42088, and NIA AG-05128).

424.7 DESENSITIZATION OF NEUROTENSIN RECEPTOR-MEDIATED CYCLIC GMP FORMATION IN NEUROBLASTOMA CLONE N1E-115. J.A. Gilbert and E. Richelson. Mayo Foundation, Rochester, MN 55905.

Murine neuroblastoma clone N1E-115 possesses receptors which specifically bind the tridecapeptide neurotensin (NT) and mediate the formation of intracellular cyclic GMP and the stimulation of inositol phospholipid hydrolysis. These cells also rapidly degrade NT in a sequential fashion (Soc. Neurosci. Abstr. 12:762, 1986). To study the effect of prolonged exposure of cells to NT on subsequent neurotensin receptor-mediated intracellular cyclic GMP formation, conditions were employed in which NT degradation is prevented: the incubation of 10^{-8} M NT with 0.1 x 10^6 intact cells in a phosphate-buffered saline solution at 37° with inclusion of the protease inhibitors 1,10-phenanthroline (1 mM) and Z-Pro-Prolinal (1 µM).

After exposure of clone N1E-115 to nondegraded NT for different lengths of time, the incubation was stopped by chilling the cellular solution and removing the cells from the incubation mixture by centrifugation. The cells were washed free of any residual NT and protease inhibitors by resuspension in buffer and collection by centrifugation. The cells were then suspended in a small volume of buffer and were kept at 4° until their distribution into Linbro multiwell plates for assay at 37°. The ability of the treated cells to respond to NT stimulation was then measured by their subsequent production of intracellular cyclic GMP (stimulation of 0.1 x 10^6 cells with 0.1 µM NT).

Neurotensin receptor-mediated cyclic GMP formation in neuroblastoma clone N1E-115 was decreased following prolonged exposure of intact cells to neurotensin. The time course of this desensitization was very rapid; neurotensin-stimulated cyclic GMP formation decreased to its lowest levels, 10-30% of control values, following exposure of intact cells to NT for only 5 min. This desensitization was specific: cells desensitized by exposure to NT demonstrated no decrease in their cyclic GMP production upon stimulation by agonists specific for other peptide receptors on these cells, i.e., angiotensin II (1 µM) or bradykinin (10^{-8} M).

Dose-response curves for neurotensin-stimulated cyclic GMP production were determined for control cells and for cells incubated with agonist for 2 or 5 min. Neurotensin preincubation with intact N1E-115 cells for increasing lengths of time caused time-dependent shifts to the right of the dose-response curve and reductions in the maximum cyclic GMP response.

Desensitization was reversible, but resensitization was a slower process than was desensitization. Recovery of full cyclic GMP production, 90-100% of control levels, was observed following incubation of the desensitized cells in buffer for a period of 10 min. (Supported by MH27692 and Mayo Foundation).

424.8 TISSUE HETEROGENEITY OF [^{3}H]NEUROTENSIN BINDING IN RATS: STUDIES WITH DYNORPHIN A 1-13, L-156,903 and LEVOCABASTINE. D.J. Pettibone, J.A. Totaro*, E. Harris* and F.M. Robinson* Merck Sharp and Dohme Research Laboratories, West Point, PA 19486

[^{3}H]Neurotensin (NT) binds to rat brain and uterus in vitro in a complex manner, exhibiting similar 'high' (Kd ~ 0.3 nM) and 'low' (Kd ~ 5 nM) affinity binding components. Competition studies with NT fragments suggest a fundamental similarity in the recognition pattern of the NT binding sites in these two tissues (Endo. Soc. Abst. #731,1987). In the present studies, however, using dynorphin A 1-13 (DYN), L-156,903 [N-oxo-3-(10H-phenothiazine-10-yl) propyl-1-arginyl-1-prolyl-1-phenylalanine] and levocabastine, clear distinctions in the NT binding sites of brain and uterus emerged.

Brain. Both DYN and L-156,903 inhibited potently [^{3}H]NT (1 nM) binding to rat brain membranes producing biphasic, shallow (nH, 0.5-0.6) competition curves indicating widely divergent affinities for the two [^{3}H]NT binding sites. The high affinity components of DYN and L-156,903 competition were similar (IC_{50}.H ~ 25 nM) whereas the low affinity components differed (IC_{50}.L: DYN ~ 1 µM; L-156,903 ~ 25 µM). Structure activity studies with these compounds indicated that the NT binding activity of DYN was due to the non-opiate C-terminus. Substitution of His for Arg or Tyr for Phe of L-156,903 reduced binding potency.

Inclusion of L-156,903 or DYN in [^{3}H]NT saturation binding assays at concentrations near the inflection points of their competition curves, reduced dramatically [^{3}H]NT binding to the 'low' affinity (5 nM) NT site with comparatively little effect on the 'high' affinity (0.3 nM) site. Similar results were found with levocabastine (5 µM), a compound which selectively blocks the 'low' affinity binding site for NT (Schotte et al., Naunyn-Schmiedeberg's Arch Pharm 333: 400, 1986). Levocabastine (5 µM) completely eliminated the high affinity binding component of DYN and L-156,903,leaving monophasic inhibition curves with IC_{50} values (1.3 µM and 20.3 µM, respectively) indicative of their low affinity components. Together these results indicate that, in the brain DYN and L-156,903, like levocabastine have high affinity and selectivity for the 'low' affinity NT site.

Uterus. DYN and L-156,903 were considerably weaker (IC_{50}'s > 100 µM) in competing with [^{3}H]NT (5 nM) binding to rat uterine membranes. Similarly, levocabastine was inactive at concentrations up to 100 µM in the uterus whereas in the brain levocabastine at 1-10 µM maximally inhibited 50-60% of the specific [^{3}H]NT binding. The marked contrast in binding activities of these compounds in brain versus uterus was evident regardless of whether brain tissue from male or female rats was used.

These results demonstrate significant differences in the binding profile of the 'low' affinity NT site between rat brain and uterus.

424.9 MODULATION OF HYPOTHALAMIC CHOLECYSTOKININ RECEPTORS WITH CHANGES IN MAGNOCELLULAR ACTIVITY. N.C.Day*, M.D.Hall*, R.G.Hill and J.Hughes* (SPON:P.J.R.Cobbett). Parke-Davis Research Unit, Addenbrookes Hospital site, Cambridge, CB2 2QB, UK.

Cholecystokinin 26-33 (CCK8) is found in high concentrations in the hypothalamus, where it is distributed amongst a number of discrete nuclei. CCK receptor distribution in the hypothalamus correlates well with peptide distribution, with high densities observed in the ventromedial, dorsomedial, paraventricular (PVN) and supraoptic nuclei (SON)(Clark et al., Br.J.Pharmacol. 90:4P, 1987). The presence of CCK receptors in the PVN and SON suggests a role for CCK in neurosecretory processes and has prompted the present investigation into the function of these receptors. Hence, PVN and SON CCK receptor distribution and density were determined by autoradiographical techniques in saltloaded and homozygous Brattleboro rats - two conditions in which the PVN/SON-pituitary axis is activated.

CCK receptor autoradiography was performed on the following experimental groups: control Sprague-Dawley (SD) rats; SD rats given 2% NaCl for 11 days; control Long Evans (LE) rats; heterozygous and homozygous Brattleboro rats. Briefly, slide-mounted sections were incubated with ^{125}I-Bolton & Hunter labelled CCK8 (^{125}I-CCK8; ~0.25nM) in HEPES buffer (10mM; pH 7.2; 25°C) for 2h. Non-specific binding was defined in the presence of unlabelled CCK8 (1uM). Sections were subsequently washed, dried and apposed to Ultrofilm for 7-14 days, followed by development of the film in Kodak D19. The resulting autoradiogram was used to produce black and white images of receptor distribution, and analysed by computer-assisted image analysis to quantify receptor density.

^{125}I-CCK8 receptor binding was greatly elevated in the PVN and SON of the saltloaded SD rat compared to control animals. Similarly, the homozygous Brattleboro rat had significantly greater levels of PVN and SON ^{125}I-CCK8 receptor binding compared to the heterozygous Brattleboro rat and LE controls. Detailed distribution studies revealed that this increase in ^{125}I-CCK8 receptor binding was localized only to the magnocellular (and not parvocellular) divisions of the PVN, a finding consistent with stimulation of magnocellular neurones in both saltloaded and Brattleboro rats. Furthermore, under the above conditions of magnocellular hyperactivity, high levels of ^{125}I-CCK8 receptor binding were observed in all the accessory magnocellular nuclei. Autoradiographic displacement studies carried out on the SON demonstrated that the increase in ^{125}I-CCK8 binding in the saltloaded rat reflected a ~230% increase in Bmax, with no change in K_d. In conclusion, CCK receptor binding site density in the PVN and SON appears to be modulated with changes in magnocellular activity - this may reflect a role for CCK in vasopressin and oxytocin release processes.

424.10 DEVELOPMENT OF A MONOCLONAL ANTIBODY AGAINST CCK-8 AND ITS EFFECT ON RADIOLIGAND BINDING AND BIOLOGICAL ACTIVITIES. C.W. Lin*, J. Chen*, D. Witte*, T. Miller*, F. Wagenaar* and A.M. Nadzan*. (SPON: W. Giardina) Neuroscience Area, Pharmaceutical Research Division, Abbott Laboratories, Abbott Park, IL 60064.

Cholecystokinin octapeptide (CCK-8) is one of several brain-gut peptides. In the CNS it is found in high concentrations in the cortex and fulfills many of the criteria expected for neurotransmitters. In the pancreas, it stimulates the release of amylase, presumably by increasing the hydrolysis of polyphosphoinositides which mobilizes intra-cellular calcium levels. Because the biochemical mechanism of CCK in the brain is poorly understood and because of the lack of specific antagonists against the central type of CCK receptors, we have developed a monoclonal antibody (LTD14.JR) against this peptide. Mice were immunized with CCK-8 conjugated to keyhole limpet hemocyanin and antibodies were screened by RIA. Spleen cells from mouse with high affinity antibody were fused with mouse myeloma. Hybridoma cultures were screened for antibody activity using EIA. LTD14.JR was identified as the candidate for culturing and ascites fluids were produced in mice. RIA determinations revealed that the purified LTD14.JR recognized the C-terminal fragments of CCK-8 with nM affinities, while N-terminal fragments of CCK-8 were inactive in the uM range. In radioligand binding assays, LTD14.JR decreased the affinity of CCK-8 by 10 fold in both the cerebral cortex and pancreas; in pancreatic acinar preparations, LTD14.JR inhibited the response of CCK-8 in the amylase and PI assays. These results indicate that LTD14.JR behaves as a CCK antagonist which should prove useful in assessing the physiological significance of CCK in the CNS.

424.11 REDUCTION OF CCK-8 BINDING IN THE NUCLEUS OF THE SOLITARY TRACT IN UNILATERALLY NODOSECTOMIZED RATS. E.E. Ladenheim, R.C. Speth and R.C. Ritter. Dept. of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164 and WOI Regional Program in Veterinary Medical Education, University of Idaho, Moscow, ID 82843.

High affinity binding sites for cholecystokinin (CCK) have been identified in the nucleus of the solitary tract (NST), area postrema (AP) and the spinal trigeminal complex (SP5) (Zarbin et al., 1983; Moran et al., 1986). Previous work from our laboratory has shown that pretreatment of rats with the neurotoxin capsaicin produces a significant depletion of CCK receptor binding in the NST and AP but not in SP5 (Ladenheim et al., 1986). Since capsaicin destroys small diameter, unmyelinated sensory neurons, the receptors in the NST and AP may be located on capsaicin-sensitive vagal afferent terminals. In order to investigate whether capsaicin-induced depletion of CCK receptor binding may be attributed to the destruction of vagal afferent neurons, we examined high affinity binding of ^{125}I Bolton-Hunter labeled CCK-8 in the brains of unilaterally nodosectomized and capsaicin-treated rats. In confirmation of our previous report we found that capsaicin pretreatment decreases the density of CCK receptor binding in the medial and commissural NST and in the AP. In addition, we found that unilateral nodosectomy markedly reduces the density of binding in the medial and commissural NST on the side ipsilateral to the transected ganglia but produces no significant depletion in the AP. CCK receptor binding on the side contralateral to the lesion does not differ from that of controls. These results indicate that the receptors located in the NST depend on intact primary vagal afferent terminals. The fact that unilateral nodosectomy causes a depletion of CCK binding ipsilaterally in these regions suggests that the receptor loss in capsaicin-treated animals is due to the destruction of vagal sensory neurons. The failure of unilateral nodosectomy to diminish CCK binding in the AP suggests that either the AP receives significant capsaicin-sensitive innervation from the contralateral vagus nerve or that the CCK receptors in this region are not vagally dependent. These data are consistent with the possibility that central CCK receptors modulate the activity of vagal afferent neurons. Supported by NIH grants R01 NS20561 and R01 NS21805.

424.12 PEPTIDE ANTAGONISTS OF CCK-8. M. Knight and T. R. Burke,Jr.*. Peptide Technologies Corporation, Trinity College, Washington, D.C. 20017

For our studies of the structure-activity relationships of CCK 1-8 or CCK 26-33 for its receptors, we have synthesized metabolically and chemically stable analogs of the N-terminal fragment heptapeptide CCK 1-7 and hexapeptide, CCK 1-6. Previously the acetylated peptide amides were found to inhibit the 0.3 nM CCK induced release of enzymes from guinea pig pancreatic acinar cells with potencies of 5-7 uM. The peptides were found to have affinities for central CCK receptors of guinea pig cortex in the range of 20 uM. Analogs with Nle substituted for Met in the peptide and with the carboxyl amide alkylated displayed greater potency in inhibiting CCK in guinea pig pancreatic secretion. Specifically, Ac Nle^{3,4} CCK 2-6 dimethylamide and Ac Nle^{3,5} CCK 2-7 ethylamide displayed potencies of 0.18 uM and 0.034 uM, respectively (R.T. Jensen). The unsulfated peptides were much less potent. These are the most potent peptide inhibitors on guinea pig pancreas to date. We are preparing more alkylamide structures of the CCK 2-7 fragment and will determine potencies in pancreatic function and central receptor binding. More potent specific inhibitors of CCK may serve to delineate the central functions of the neuropeptide.

424.13 ANTAGONISM OF CHOLECYSTOKININ-INDUCED EFFECTS ON NEURONS IN THE RAT NUCLEUS ACCUMBENS BY LORGLUMIDE. Richard J. Kasser, Xiu-Ti Hu, and Rex Y. Wang, Dept. of Psychiatry and Behavioral Science, SUNY Stony Brook, Stony Brook, NY 11794.

Makovec et al., (Arzneim.-Forsch. Drug Res. 36:98-102, 1986) reported that lorglumide (CR-1409, Rotta Labs, Monza, Italy), a new glutaramic acid derivative, potently inhibits cholecystokinin (CCK) binding activity in rat pancreatic acini and mouse cerebral cortex. Our lab has previously shown that iontophoresis of proglumide (0.1 M), a prototypical CCK antagonist, effectively blocks CCK-induced excitation in the medial part of the nucleus accumbens (NAc) (White and Wang, Brain Res. 300:161-166, 1984) which receives topographical CCK/dopamine (DA) projections from the ventral tegmental area (VTA or A10). However, proglumide has a low affinity for CCK receptor sites. The purpose of the present study was to determine whether lorglumide is a potent and selective antagonist of CCK-induced excitation in rat NAc by using single unit recording and microiontophoretic techniques.

Rats were anesthetized with chloral hydrate and mounted in a stereotaxic apparatus. NAc cells were recorded using standard electrophysiological techniques.

As previously reported (White and Wang, 1984), many NAc cells were quiescent. Therefore, the effects of CCK and lorglumide were studied on glutamate (GLUT)-induced as well as spontaneous neuronal activity. Iontophoretically applied CCK activated a subpopulation of both quiescent and spontaneously active cells and, as previously described (Wang and Hu, Brain Res. 380:363-367, 1986), markedly potentiated the activity evoked by GLUT. Iontophoretically applied lorglumide (1 μM) but not NaCl (20 μM) blocked CCK-induced activation (n=13) and CCK potentiation of GLUT-induced activation of NAc cells (n=15). Intravenous lorglumide (0.3 μg/kg) also effectively prevented the excitatory effects induced by CCK (n=8). Iontophoresis of 1 μM lorglumide failed to block the excitatory effect evoked by GLUT or the inhibitory effects produced by DA (n=4) and neuropeptides such as neurotensin (n=6), substance P (n=6) and somatostatin (n=7) on NAc cells, suggesting that the antagonistic action of lorglumide on CCK-induced effects is selective. However, ionto-lorglumide at high concentration (1 mM), in addition to blocking CCK-induced effects, potentiated GLUT-induced excitation in 7 cells. Lorglumide never potentiated CCK-induced activity.

In conclusion, the results of the present study indicate that lorglumide is at least 10,000 times more potent than proglumide in blocking CCK-induced effects on NAc cells. If the finding can be extended to other brain areas, lorglumide has the potential to be the CCK antagonist of choice for use in the CNS. (Supported by USPHS Grants MH-41440, 41696 and 00378 to RYW).

424.14 PRESENCE OF [^{3}H]NEUROPEPTIDE Y BINDING SITES IN HUMAN BRAIN. J.C. Martel, R. Alagar*, S. St-Pierre* and R. Quirion. Departments of Psychiatry, Pharmacology and Therapeutics and Douglas Hospital Research Center, McGill University, Montreal, Canada and Institut National de la Recherche Scientifique en Santé, Pointe-Claire, Canada.

In man, high amount of neuropeptide Y (NPY)-like immunoreactivity is present in the hypothalamus, thalamus, striatum, nucleus accumbens, amygdala, septum and throughout the cortex and hippocampus. However, the presence of specific NPY receptors in human brain has yet to be reported. Recently, we found that in rat brain, high densities of NPY binding sites are concentrated in the olfactory bulb, cortex, lateral septum, thalamus, hippocampus and area postrema (Martel et al., Peptides 7:55, 1986). We report here on the distribution of [^{3}H]NPY binding sites in human brain using membrane preparations of microdissected brain areas. Brains were obtained at autopsy with postmortem delays ranging between 7 and 32 hrs. One half of the brain was fixed for diagnosis purpose. The other half was cut into approximately 1 cm thick coronal sections and frozen in 2-methyl butane at -40°C and stored at -80°C. Microdissection was made on a cold plate using appropriate atlases. Membranes were prepared by homogenization in Krebs-Ringer buffer (1:40 w/v), and centrifugation at 35,000 x g for 15 min at 4°C. The pellets were rinsed, resuspended and centrifuged again. Final pellets were rinsed and resuspended to give a protein concentration of 12-18 mg proteins/ml of membrane preparations. For binding assay 100μl of this preparation were incubated at 25°C for 90 min in a final volume of 500μl of Krebs Ringer containing 0.1% (w/v) bovine serum albumin, 0.05% (w/v) bacitracin and concentrations of [^{3}H]NPY (Amersham, 70 Ci/mmol) ranging from 0.05 nM to 16 nM. Incubations were terminated by rapid filtration (plus 3 x 5 ml rinses with ice cold buffer) through #32 glass filters (Schleicher & Schuell)(presoaked in 1% polyethyleneimine for 2 hrs) using a cell harvester filtering apparatus (Brandel). Specific binding was calculated as the difference in radioactivity bound in absence and presence of 0.5 μM NPY. In human brain, [^{3}H]NPY apparently binds to a single class of high affinity sites (Kd range: 0.7-3.5 nM). The highest densities of binding sites were observed in the putamen (Bmax= 49 fmol/mg protein) followed by the caudate nucleus (Bmax= 36 fmol/mg protein), cingulate gyrus (Bmax= 34 fmol/mg protein), superior frontal gyrus (Bmax= 27 fmol/mg protein) and prefrontal cortex (Bmax= 15 fmol/mg protein). These preliminary data suggest that [^{3}H]NPY binding sites are discretely distributed in human brain. We are currently investigating their possible alteration in various neurological diseases (Supported by an MRCC grant to RQ).

424.15 EFFECT OF NEUROPEPTIDE Y ON ELECTRICALLY STIMULATED RELEASE OF ^{3}H-NE FROM RAT HYPOTHALAMIC SLICES. H. Yokoo[1], D. Schlesinger[2] and M. Goldstein.[1] Department of Psychiatry, Neurochemistry Research Laboratories[1] and Cell Biology,[2] New York University Medical Center, NY, NY 10016.

The finding that neuropeptide Y (NPY) is colocalized with norepinephrine (NE) and epinephrine (E) in brain stem cell groups which send efferent fibers to the paraventricular nucleus (PVN) of the hypothalamus prompted us to investigate its effect on spontaneous and electrically stimulated release of ^{3}H-NE from hypothalamic slices (H-S). H-S were incubated at 37°C with ^{3}H-NE for 20 min and superfused at the rate of 1 ml/min for 60 min with Krebs Ringer Buffer solution. At the end of this period samples were collected every 5 min and electrical stimulation (20 mA, 2 msec at 3 Hz) was applied at 70 min (S_1) and at 120 min (S_2) following initiation of superfusion. In presence of tested drugs the superfusion was started 20 min prior to S_2 and continued until the end of the experiment. The radioactivity in the collected samples and in the tissues was determined by liquid scintillation spectrometry. The spontaneous ^{3}H-NE release from H-S was not significantly altered by NPY. However the release of ^{3}H-NE from the electrically stimulated HS was inhibited by NPY in a dose-dependent manner. The S_2/S_1 ratio decreased from the control value of 0.845 ± 0.01 to 0.689 ± 0.03 and 0.563 ± 0.04 in presence of 10^{-9}M and 10^{-8}M NPY, respectively.

To determine the effects of α_2 adrenoceptors on NPY-induced inhibition of ^{3}H-NE release from H-S, we have investigated the inhibitory effects in presence of the α_2 adrenoceptor agonist UK 14,304 and the α_2 adrenoceptor antagonist RX 780194. The inhibitory effects of UK 14,304 on the electrically stimulated release of ^{3}H-NE were potentiated by NPY at concentrations of 10^{-9}M-10^{-8}M. Furthermore, In presence of RX 780194 (10^{-8}M) the inhibitory effects of NPY were significantly reduced. These results suggest that the inhibitory effects of NPY on ^{3}H-NE release in H-S are mediated in part by α_2 adrenoceptors. NPY has no inhibitory effects on electrically stimulated release of ^{3}H-NE from cerebral cortical slices, a region apparently devoid of NE/NPY coexistent neurons. Thus, our findings support the idea that NPY might be involved in the regulation of neurotransmision in NE/NPY and/or E/NPY coexistent neurons of the hypothalamus. These studies were supported in part by NIMH Grant 02717 and NIH Grant 06801.

424.16 REGULATION OF ANGIOTENSIN II RECEPTORS IN BRAIN AND ADRENAL AFTER REPEATED SODIUM DEPLETION. R.R. Sakai*, A.N. Epstein, R.R. Miselis & S.J. Fluharty. (SPON: S.P. Frankmann) Depts. of Animal Biology & Biology, Institute of Neurological Sciences, Univ. of Pennsylvania, Phila. PA 19104.

During sodium depletion angiotensin II (ANG II) appears to act centrally to arouse sodium appetite and peripherally to release aldosterone. ANG II binding sites are upregulated in the adrenal during sodium deprivation, while a similar response within the brain is controversial. The present report examines the number and affinity of ANG II binding sites in these two target organs after repeated sodium depletions and correlates this receptor analysis with changes in sodium ingestion and aldosterone release.

Male SD rats on a sodium deficient diet for 1 day were treated with furosemide (10 mg, sc) once a week for 4 successive weeks. After each depletion water and 3% NaCl ingestion was monitored daily and, in separate animals, blood was collected for aldosterone RIA. One week after the 4th depletion, when they were sodium-replete, the animals were sacrificed and the adrenal glands and hypothalamus-thalamus-septum (HTS) region of the brain were removed and membranes prepared for binding assays. HTS membranes were incubated with at least 7 concentrations of (^{125}I)-ANG II (0.025 - 1.0 nM) at 25°C for 60 min. Saturation analysis of adrenal membranes was performed with at least 10 concentrations of (^{125}I)-ANG II (0.10 - 5.0 nM). In both cases bound radioligand was separated by vacuum filtration and non-specific binding was defined with 1 uM ANG II.

After the fourth depletion the Bmax of ANG II receptors in the HTS increased by 42% (6.1 ± 0.4 to 8.7 ± 0.4 fmols/mg prot) without a change in K_D (362 ± 24 vs 410 ± 32 pM). This elevation of binding capacity was correlated with elevated sodium intake. Similarly, the Bmax of adrenal ANG II receptors increased (208 ± 27 to 434 ± 37 fmols/mg prot) while the K_D was not altered (0.91 ± 0.15 vs 1.03 ± 0.17 nM). This upregulation was associated with a pronounced increase in plasma aldosterone levels during the fourth (1833 ± 89 pg/ml) but not previous depletions (886 ± 78 pg/ml).

Collectively, these results suggest that an upregulation of ANG II receptors in brain and adrenal may be involved in the mobilization of complementary physiological and behavioral responses to repeated sodium depletion. Supported by NS 23986, GM 27739, NS 03469, MH 17168 and the University of Pennsylvania Research Foundation.

424.17 REGULATION OF BRAIN ANGIOTENSIN II RECEPTORS DURING DEHYDRATION. A.J. Nazarali*, J.S. Gutkind* and J.M. Saavedra (SPON: G.B. Baker) Section on Clinical Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland, USA.

Cerebral structures primarily located in the forebrain have been implicated in the regulation of body fluid homeostasis. Increasing evidence points to the two anatomically connected circumventricular organs, the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT) as the regions important for mediating responses to dipsogenic stimuli.

The principal peptide responding to water deprivation is angiotensin II (ANG II). We have therefore investigated binding sites for ANG II in distinct rat brain nuclei. Male Sprague-Dawley rats (225-250g) were used in this study. The animals were kept in a temperature-controlled room (24 ± 1°C) with a 12hr-on/12hr-off lighting schedule and housed in standard laboratory metal cages. Laboratory food and water were provided ad libitum to one set of animals (N=8,control group) whereas only food was provided to the second set of animals (N=8, treatment group). The treatment group of animals were dehydrated for a period of 5 days. All animals were killed by decapitation between 09.00 and 11.00 hr after 5 days. Brains were rapidly dissected out, meninges removed, and the brain tissue immediately frozen at -70°C in isopentane on solid carbon dioxide. Receptors for ANG II in brain nuclei were quantified by incubating 16 µm brain sections with a saturating concentration of 3 nM [^{125}I]Sar^{1} ANG II followed by autoradiography with computerized microdensitometry and comparison with ^{125}I-standards. Binding densities in fmol/mg (mean ± S.E.M.) obtained in the various brain nuclei for control (C) and water deprived (D) animals were respectively: SFO, 171 ± 12 (C) and 373 ± 10 (D); paraventricular nucleus (PVN), 116 ± 12 (C) and 136 ± 11 (D); area postrema (AP), 140 ± 8.9 (C) and 136 ± 10 (D); nucleus of the solitary tract (NTS), 166 ± 8 (C) and 171 ± 9 (D);and OVLT, 185 ± 15 (C) and 210 ± 20 (D).

Our results show a significant ($p < 0.05$, Student's t-test) up-regulation of ANG II receptors only in SFO after water deprivation. Although a trend for higher numbers of binding sites for ANG II was observed in OVLT, this was however not significant. Other brain nuclei investigated showed no significant differences ($p > 0.05$). These findings are of anatomical and functional significance implicating the SFO and ANG II receptors in the regulation of body fluid homeostasis.

424.18 INCREASED NUMBER OF ANGIOTENSIN II RECEPTORS IN SELECTIVE BRAIN NUCLEI OF DOCA-SALT HYPERTENSIVE RATS. J.S. Gutkind* and J.M. Saavedra (SPON: F.M.A. Correa), Section on Clinical Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892, USA.

Angiotensin (ANG) is a circulating peptide involved in the peripheral regulation of water and salt metabolism and cardiovascular function. In addition, ANG has actions in the brain through stimulation of specific receptors in circumventricular organs, and in brain areas protected by the blood brain barrier.

The central ANG system has been reported to be activated in the DOCA-salt experimental model of hypertension. We therefore compared the concentration of ANG receptors in specific brain nuclei of control (C) (n=8), DOCA treated (D) (DOCA pivalate, 25 mg/kg s.c. weekly, n=8), salt treated (S) (1.0 % NaCl in drinking water, n=8) and DOCA-salt treated (DS) (DOCA + salt treatments, n=8) male Wistar Kyoto rats. All animals were uninephrectomized.

After 4 weeks of treatment, only the DOCA-salt group showed increased systolic blood pressure (179 ± 5 mmHg DS vs 106 ± 3 mmHg C, $p<0.01$). All animals were killed by decapitation between 09.00 and 11.00 hrs, the brains were rapidly dissected and immediately frozen in isopentane (-30°C). Binding sites for ANG II in brain nuclei were quantified by incubating 16 µm brain sections with a 3 nM saturating concentration of [^{125}I]Sar^{1}ANG II followed by autoradiography with computerized microdensitometry and comparison with [^{125}I]-standards.

ANG receptors were significantly increased in selective brain areas of DOCA-salt hypertensive rats. In those areas, binding densities, in fmol/mg protein were: median preoptic nucleus, 131 ± 9 (C) and 182 ± 6 (DS); subfornical organ, 130 ± 8 (C) and 201 ± 8 (DS); paraventricular nucleus, 78 ± 6 (C) and 149 ± 6 (DS) ($p<0.01$); area postrema, 73 ± 7 (C) and 125 ± 10 (DS); nucleus of the solitary tract, 81 ± 6 (C) and 151 ± 10 (DS) ($p<0.05$). Binding site density was not different in suprachiasmatic nucleus: 124 ± 14 (C) and 128 ± 18 (DS). Salt treatment alone increased ANG binding in median preoptic nucleus, subfornical organ, paraventricular nucleus and nucleus of the solitary tract. However, in the subfornical organ, paraventricular nucleus and nucleus of the solitary tract, the alteration in ANG receptors induced by DOCA-salt were significantly higher than those produced by salt treatment.

Our data indicate that ANG receptors are altered in central areas related to blood pressure control and fluid homeostasis in DOCA-salt hypertensive rats, and may be involved in the pathophysiology of this experimental model of hypertension.

424.19 IS PHOSPHATIDYLINOSITOL A SECOND MESSENGER FOR CNS BOMBESIN-LIKE PEPTIDES? Terry W. Moody and V.N. Hari Prasad* . Dept. Biochemistry, The George Washington Univ. Med. Sch., Washington, D.C. 20037.

Bombesin (BN)-like peptides and receptors are discretely distributed in the rat CNS where they may regulate neural activity (Zarbin et al., J. Neurosci., 5: 429, 1985). While BN causes hypothermia, hyperglycemia, grooming, satiety and altered hormone secretion in rats its second messenger remains unkown. Here we investigated if BN-like peptides altered second messenger production in the rat CNS.

Rat brain slices (250 µm) were prepared as described previously (Fremeau et al., J. Neurochem., 46: 1947, 1986) and rinsed in Kreb's buffer gassed with 95% O_2/5% CO_2 at 37°C for 30 min. The slices were then incubated with 1 µM BN or the structurally related gastrin releasing peptide (GRP) for 30 min. Cyclic AMP and cGMP were then assayed by radioimmunoassay. While VIP (1 µM) elevated the cAMP levels 4-fold, BN or GRP had no effect on the cAMP or cGMP levels. Because BN receptors may not be coupled to adenylate or guanylate cyclase we investigated if BN altered phosphatidylinositol$_3$ turnover. The rat brain slices were preincubated with ^{3}H-inositol (3 µCi) for 30 min in the presence of LiCl (7.5 mM) and then 1 µM BN or GRP was added and incubated for 60 min. The reaction was terminated as descibed previously (Berridge et al., Biochem. J., 206: 587, 1982) and the ^{3}H-inositol-1-phosphate was determined using column chromatography techniques. Carbachol (1 mM), BN (1 µM) and GRP (10 µM) routinely stimulated phosphatidyl inositol production by 300, 50 and 20% respecively relative to controls. The effect caused by BN was dose dependent. The increase in phosphatidylinositol turnover was greatest using slices derived from the olfactory bulb followed by the hippocampus, striatum, frontal cortex, thalamus and midbrain. Because those areas which strongly stimulate phosphatidylinositol turnover have high receptor binding activity, CNS receptors for BN-like peptides may utilize phosphatidylinositol as a second messenger. Supported by NSF grant BNS-8500552.

424.20 LABELING OF BOMBESIN RECEPTORS IN MEMBRANES FROM A SECRETORY CELL LINE BY PHOTOAFFINITY CROSSLINKING. J.B. Fischer and A. Schonbrunn. Lab. of Toxicology, Harvard Sch. of Pub. Health, Boston, MA 02115.

The bombesin-like peptides, which include the gastrin-releasing peptides (GRPs) and neuromedins B and C, have many biological actions, including the stimulation of hormone secretion from the pituitary and pancreas. High affinity binding sites for the bombesin-like peptides have been characterized in several target tissues. However, although these binding sites probably mediate the biological actions of mammalian bombesins, little is known about the molecular properties of these putative receptors. We have begun to biochemically characterize bombesin receptors by covalently linking specifically-bound [^{125}I]GRP$_{14-27}$ to its binding site using bifunctional crosslinking reagents. GRP$_{14-27}$ was iodinated by the lactoperoxidase-glucose oxidase method and purified by HPLC using a triethylammonium phosphate buffer system with an acetonitrile gradient. Membranes were prepared from HIT-T15 cells, an insulin-secreting cell line derived from hamster pancreas in which bombesin acts as a powerful secretagogue. [^{125}I]GRP$_{14-27}$ binds to HIT cell membranes with high affinity, and is displaced by bombesin, GRP, GRP$_{14-27}$, and neuromedins B and C at nanomolar concentrations but not by micromolar concentrations of unrelated peptides. In the crosslinking experiments, 0.1-0.3 nM [^{125}I]GRP$_{14-27}$ was bound to the membranes at 37°C for 60 min. The membranes then were cooled to 4°C to slow the dissociation of the bound ligand and incubated with 100 µM N-5-azido-2-nitrobenzoyloxy-succinimide (ANBNOS) in the dark for 30 min, followed by UV irradiation for 1 min. After washing once by centrifugation, the membranes were solubilized in electrophoresis sample buffer, and analyzed by polyacrylamide gel electrophoresis in the presence of 2-mercaptoethanol. Autoradiograms of the dried gels revealed the labeling of one broad band with a molecular weight of 61-75 kDa. Other crosslinking reagents, including some which do not require UV irradiation, resulted in labeling of a band that was essentially identical to that labeled with ANBNOS. The labeling of this band was completely blocked by nanomolar concentrations of unlabeled bombesin in a dose-dependent manner, in parallel with bombesin inhibition of reversible binding. The addition of a mixture of protease inhibitors to the membrane preparation and incubations (phenylmethylsulfonyl fluoride, benzamidine, leupeptin, soybean and lima bean trypsin inhibitors, and pepstatin A) did not change the molecular weight of the labeled band. However, treatment of the labeled membranes with endoglycosidase F, which cleaves N-linked carbohydrates from proteins, decreased the molecular weight of the labeled band, suggesting that it is a glycoprotein. We conclude that a 61-75 kDa glycoprotein is a major component of the bombesin receptor in HIT cells.

425.1 HYPOTHALAMIC GnRH NEURON-PITUITARY GONADOTROPE FUNCTION THROUGHOUT POSTNATAL DEVELOPMENT IN THE GUINEA PIG: AND A COMPARISON WITH THE PRIMATE. M.O. Fraser* and T.M. Plant, Department of Physiology, University of Pittsburgh School of Medicine, and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

The protracted delay in the onset of sexual maturation in primates appears to be occasioned by interruption of intermittent hypothalamic GnRH secretion from shortly after birth until the time puberty is initiated. This prepubertal hiatus in GnRH release is not imposed by the inhibitory action of sex steroids, since a similar developmental pattern in GnRH secretion, as inferred from the time course of circulating LH levels, is observed in the absence of gonadal feedback. That the guinea pig may exhibit an analogous mechanism for the restraint of the reproductive axis during prepubertal life has been proposed by Nass et al. (Endocrinology 115:220, 1984). These authors reported that ovariectomy in 10 day old guinea pigs did not result in an immediate rise in gonadotropin secretion. Instead, circulating LH was maintained at concentrations comparable to those of intact controls until approximately 30 days of age. The foregoing findings prompted the present study, which was designed to describe for the guinea pig the timing of the onset of the non-gonadal prepubertal restraint of LH secretion previously observed by Nass et al. To this end, 2 day old English Short Hair guinea pigs of each sex were either castrated under metofane anesthesia (n = 8/group), sham castrated (n = 2/group), or left intact (n = 8/group). Serum samples (800 µl) were collected by cardiac puncture under metofane anesthesia on day 2 of age, immediately prior to surgery, and thereafter at 4 day intervals until 98 days of age. Animals remained with their mothers throughout the duration of the experiment. Serum LH was determined by a RIA system utilizing intact male guinea pig anterior pituitary extract as standard, anti-ovine LH antiserum (GDN-15) as 1st antibody, and radioiodinated rat LH (125I- NIH-rLH-I-7) as trace. In both sexes, castration on day 2 resulted in an immediate increase in LH secretion as reflected by a progressive rise in circulating concentrations of this gonadotropin which plateaued between 34-42 days of age at levels 10 fold higher than those in intact controls. In contrast to the earlier findings, our results suggest that the guinea pig does not exhibit a prepubertal restraint of GnRH secretion analogous to that seen in primates. The contradiction in results obtained in the two studies is probably due to differences in experimental design. Unlike the present studies, in which young guinea pigs remained with their mothers throughout, Nass et al. weaned their pups at 10 days of age (the day of ovariectomy), and subsequently housed them in isolation from their mothers. Since separation of mother and infants may be anticipated to elicit a state of stress in the guinea pig pups, and since stress is known to suppress gonadotropin secretion, the delay in the postcastration rise in LH secretion observed by Nass et al. most likely reflects a concomitant activation of the hypothalamic-pituitary-adrenal axis rather than operation of a specific pubertal control system. However the case may be, the present study fails to provide evidence for the view that the postnatal ontogeny of GnRH release is similar in primates and guinea pigs. Supported by NIH grants HD 13254 and MH 18273.

425.2 SEASONAL PLASTICITY IN SYNAPTIC INPUT TO LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) NEURONS IN THE SHEEP F.J. Karsch*, M.N. Lehman and A.J. Silverman (SPON: G. Adamek) Develop. & Reprod. Biol., Univ. Mich., Ann Arbor, MI; Dept. Anat. & Cell Biol., Univ. Cincinnati Coll. Med., Cincinnati, OH; Dept. Anat. & Cell Biol., Columbia Univ. Coll. Phys. & Surg., New York, NY.

LHRH neurons and their projections constitute the final common pathway of a neural pulse generator which controls episodic secretion of LH. In the Suffolk ewe, photoperiodic and hormonal signals act upon the LHRH pulse generator to coordinate an annua rhythm in reproduction. Based on earlier light microscopic studies (Lehman et al., J. Comp. Neurol., 244:19), we hypothesized that structural plasticity of LHRH neurons or their synaptic inputs may in part underlie seasonal breeding the ewe. To test this we compared the synaptic inputs of LHRH neurons in adult intact ewes perfused during the breeding season, specifically during the mid-luteal phase of their estrous cycle (n=5), with those of ewes perfused during anestrus (n=5). Brain were fixed with 4% paraformaldehyde with 0.2% glutaradehyde, and processed for EM immunocytochemistry as before (Silverman & Witkin, J. Histochem. Cytochem., 33:69). Sections were silver-gold intensified prior to osmication, dehydration, and flat embedding. Sixty-five preoptic LHRH cells and 60 non-immunoreactive (non-IR) neurons from the same sections were analyzed (approx. 6 LHRH and 6 non-IR cells per animal). The mean length and percentage of plasma membrane bearing synaptic modifications (either synaptic cleft or density) were calculated

While LHRH neurons in both breeding season and anestrous ewes were relatively underinnervated compared to non-IR neurons, the mean percentage of LHRH membrane bearing synaptic modifications in breeding season ewes (1.16% ± 0.81; 4583 µm membrane examined was more than twice that in anestrous ewes (0.44% ± 0.05; 5286 µ membrane examined) ($p<.0005$; two-tailed test). This difference reflected an increase in synaptic density of both axosomatic and axodendritic inputs to LHRH neurons. In contrast, the mean percentage of membrane occupied by synaptic modifications for non-IR cells did not significantly differ between the breeding season (2.65% ± 0.49; 1601 µm membrane examined) and anestrus (2.14% ± 0.37; 1881 µm membrane examined). The mean length of synapses onto either LHRH cells or non-IR cells remained constan between the breeding season (LHRH cells; 0.29 µm ± 0.02; non-IR cells: 0.30 µm ± 0.03) and anestrus (LHRH cells: 0.27 µm ± 0.02; non-IR cells: 0.28 µm ± 0.01). In summary, preoptic LHRH cells during the breeding season possess a significantly greater density of synaptic input than during anestrous. Further experi ments are needed to determine whether this change in synaptic innervation is driven by photoperiodic and/or hormonal signals. [Supported by NIH HD 18337 (FJK) HD 21968 (MNL) & HD 10665 (AJS)

425.3 α_2-ADRENORECEPTOR BLOCKADE DIFFERENTIALLY AFFECTS STIMULATION OF LH AND FEEDING IN RESPONSE TO NEUROPEPTIDE Y (NPY). L.G. Allen*, W.R. Crowley[1] and S.P. Kalra, (SPON: J. Rabii) Dept. OB-Gyn, Univ. Fla. Coll. Med., Gainesville, FL 32610 and [1]Dept. Pharmacol., Univ. Tenn. Coll. Med., Memphis, TN 38163.

Intraventricular (Ivt) administration of NPY readily stimulates LH release and feeding in a dose-related fashion in ovarian steroid-primed ovariectomized (OVX) rats. Since there is evidence of coexistence of adrenergic transmitters with NPY and both adrenergic transmitters and NPY stimulate LH release and feeding behavior, we studied the interaction of α_2-adrenergic receptors in mediating these NPY-induced responses. Rats were OVX and received permanent cannulae in the third ventricle of the brain. Two weeks later, they were injected with estradiol benzoate (30µg) and progesterone (15mg) on day 0. On day 2, yohimbine (YOH, 3.5mg/kg, N=6) or vehicle (dH_2O, N=5) was injected i.p. and 1.5 h later these rats received either 3 µl saline or 470 pmol NPY in 3 µl saline Ivt. NPY stimulated food intake in dH_2O-treated rats. They ate 2.07 ± 0.21 g food in 60 min. The food intake was significantly ($P<0.05$) potentiated in response to NPY in YOH-treated rats, which ate 3.37 ± 0.3 g in 60 min. Similarly, NPY stimulated LH release in dH_2O-injected control rats. However, in contrast to the potentiation of feeding response, YOH pretreatment significantly ($P<0.05$) decreased the magnitude and duration of LH increase in response to NPY. In control rats, LH levels were elevated for 60 min. after NPY administration, while in YOH-treated rats LH increments were attenuated and lasted only 10 min. Since α_2-adrenoreceptor blockade potentiated NPY-induced feeding, we explored further the role of α_2-adrenoreceptors in food intake. YOH was administered in doses of 0.437, 0.875 and 3.5 mg/kg i.p. to intact female rats. Surprisingly, YOH (3.5 mg/kg) on its own stimulated feeding (0.98 ± 0.18 g,N=6) which was significantly higher than the spontaneous feeding displayed by dH_2O-treated controls (0.08 ± 0.05 g,N=6). Rats treated with 0.437 mg/kg YOH ate 0.5 ± 0.183 g (N=6) and rats treated with 0.875 mg/kg ate 0.616 ± 0.183 g (N=6, $P>0.05$ with respect to dH_2O-treated controls). These findings suggest that α_2-adrenoreceptors may mediate the neuroendocrine and behavioral responses evoked by NPY, but the mode of involvement may be different. Since the blockade of α_2-adrenoreceptors rapidly induced a moderate increase in food intake, and NPY-induced feeding was enhanced by YOH pretreatment, it implies that NPY-induced feeding, in part, may involve blockade of an adrenergic component that has previously been shown to inhibit feeding. On the other hand, stimulation of LH release by NPY may require intact excitatory α_2-adrenergic receptor function. (Supported by NIH DK 37273, HD 08634 and HD 13703).

425.4 LOCALIZATION OF PRO-GONADOTROPIN-RELEASING HORMONE (proGnRH) AND GnRH mRNA IN THE HYPOTHALAMUS OF THE RAT. M.J. Kelly, C.E. Roselli, M.A. Bosch*, J.P. Adelman* and O.K. Ronnekleiv. Dept. of Physiology and IABR, OHSU, Portland, OR 97201

Recently, the structure of the cDNAs encoding human and rat proGnRH have been derived, and the corresponding peptide sequences ascertained (Adelman et al. Proc. Natl. Acad. Sci. 33:179, 1986). Moreover, immunostaining with a specific antiserum to proGnRH (ARK-1) revealed that proGnRH is processed to GnRH in the cell body in the macaque hypothalamus (Ronnekleiv et al., Neuroendocrinology 45(6), 1987). Presently, we are reporting on the localization of GnRH mRNA and proGnRH in the hypothalamus of the rat. ARK-2 antiserum was generated against the bridging site for proGnRH, amino acids 6-16. Under RIA conditions, ARK-2 bound 20-25% of ^{125}I-proGnRH 6-16 at a 1:20,000 dilution. A standard curve using 1.0-1,000 pg proGnRH 6-16 yielded a sensitivity of 10 pg and an ED_{50} of 80 pg. Under RIA conditions, ARK-2 did not recognize GnRH or several different fragments of the decapeptide, and had only a 0.02% cross-reactivity with rat GnRH-associated peptide (GAP). Using this antibody, we measured proGnRH in acetic acid extracts of hypothalami pooled from 5-6 rats. Serial dilutions of the extracts produced inhibition curves that were parallel to the standard curve. To demonstrate the localization of GnRH mRNA, proGnRH and GnRH in rat brain, Avidin-Biotin immunohistochemistry was performed on vibratome sections through the preoptic-basal hypothalamus (POA-BH) from both males and females. This procedure was followed by in situ hybridization with the full length probe to GnRH mRNA. Cells containing GnRH were localized as described previously, and the same cells also contained the mRNA for GnRH. Moreover, the ARK-2 antiserum, diluted 1:3000, stained cells with similar distribution to GnRH. However, immunoreactive proGnRH was not present in fibers in the POA-BH or in nerve terminals in the ME-region. Preabsorption of the ARK-2 antiserum with bacterial-derived proGnRH or proGnRH 6-16 eliminated all immunostaining. The staining was not eliminated by preabsorption with GnRH, proGnRH 1-13 or rat GAP. These data indicate that proGnRH is present mostly within cell bodies in the POA-BH of male and female rats, and that some processing of proGnRH to GnRH occurs in the cell soma before transport to the nerve terminal.
(Supported by PHS grants HD16793, HD19905 and AM16879)

425.5 ELECTROPHYSIOLOGICAL EFFECTS OF SELECTIVE OPIOID AGONISTS ON HYPOTHALAMIC NEURONS IN THE FEMALE RAT. M.D. Loose* AND M.J. Kelly (SPON. J. Nutt), Dept. Physiology, OHSU, Portland, OR 97201

The mediobasal hypothalamus of the mammal controls several neuroendocrine systems related to reproduction via the neurosecretion of peptides and amines into the portal blood. Since opioids affect the reproductive system and the arcuate nucleus and cell-poor zone (ARC-CPZ) contain the highest density of opioid neurons in the hypothalamus, we began an investigation of the electrophysiological effects of selective opioid agonists on the excitability of ARC-CPZ neurons. Coronal hypothalamic slices were prepared from female rats on the morning of proestrous and maintained as previously described (Kelly et al., Brain Res. 345:264, 1985). Stable extracellular recordings could be obtained up to 12 hours after tissue preparation. KCl-filled microelectrodes (40-70 MΩ) were positioned under visual control in the ARC-CPZ. A stable baseline firing rate was obtained for at least 5 minutes before DAGO, a opioid agonist selective for the μ receptor subtype; naloxone (NAL), a specific opioid antagonist; or methoxamine (MX), a noradrenergic α_1 receptor agonist, were applied to the slice either by addition to the perfusion medium or by ejection from a pressure pipette. Excitation and inhibition by a drug were defined as a consistent and reversible change of 30% from the baseline firing rate. A dose response curve was generated for concentrations of DAGO between 1 and 100 μM (N=24). Application of 2μl of 100 μM DAGO inhibited the majority (80%) of the neurons tested. Ten μM DAGO (2 μl) caused an inhibition in 43% of the ARC-CPZ cells tested, and 1μM DAGO was ineffective when applied via the pressure pipette or in the medium. Only one cell was excited by DAGO. NAL (10 μM) did not cause any changes in the baseline firing frequency, but it did reverse the inhibitory effects of DAGO. MX (1μM) did not effect the firing of 5 out of 6 ARC-CPZ neurons, but the one cell excited by MX changed from an irregular to a bursting pattern of firing and was subsequently inhibited by 100 μM DAGO. These results suggest that activation of opioid receptors inhibits the majority of ARC-CPZ neurons in the proestrous female rat which is consistent with the inhibitory actions of the opioids on the reproductive cycle of the mammal. Experiments are in progress to further characterize the electrophysiological response to DAGO and other selective opioid agonists and to determine whether the opioid effects are altered by steroids. (Supported by PHS grant HD 19905)

425.6 BRAIN-DIRECTED DELIVERY OF ESTRADIOL PROLONGED THE OVULATION-BLOCKING ACTION OF ESTRADIOL IN RATS. D.K. Sarkar, S.J. Friedman*, S.S.C. Yen* and R. Roy*. Dept. of Reproductive Medicine, Univeristy of California, San Diego, La Jolla, CA 92093, USA

The biological potency of a chemical delivery system for estradiol (E) that involves attachment to a dihydropyridine pyridinium salt redox carrier complex was studied. Administration of this carrier estradiol complex (CE), and E (0.13-38.5 μM/kg, i.v.) in cyclic female rats lengthened the period of the estrous cycle in a dose-dependent fashion. Interestingly however, CE was more effective than an equimolar dose of E, at each of the doses examined. The minimum effective dose of CE that delayed ovulation for one estrous cycle was 0.128 μM/kg, whereas a dose of 2.56 μM/Kg of E was required to delay ovulation for 4 days. The maximum dose of CE (38.5 μM/Kg) used in this study lengthened the cycle from 4 days to 32 days. An equimolar dose of E prolonged the cycle for only 18 days. The lengthening of the estrous cycle days following the steroids could not be attributed to the direct action of the steroids on the vaginal orifice, since these rats showed no ova in the oviduct, no freshly formed corpora lutea in the ovaries and no cyclic gonadotropin release from the pituitary for a sustained period of time. When the secretion of the hypothalamic LHRH and the pituitary sensitivity to LHRH of the steroid-treated rats were examined, these rats showed significantly reduced secretion of hypothalamic LHRH but not significant alteration of the pituitary sensitivity to LHRH after 24 h of the treatment. Measurement of the brain and plasma levels of free E following CE and E treatment in ovariectomized rats revealed that the brain and plasma clearance of CE is much slower than E. When the plasma LH concentrations of CE and E-treated rats were compared, CE-treated rats showed reduced LH levels for a significantly longer period of time than E-treated rats. Furthermore, when the kinetics of the biological action and the brain hormone concentrations were compared, the levels of brain E negatively associated with the levels of plasma LH. These results suggest that the chemical delivery system for E increased the biological potency of the steroid. The increased biological potency of the CE appeared to be related to a sustained storage and release of E from the CE into the brain causing chronic inhibition of the neuroendocrine mechanism responsible for ovulation. (Supported by a grant from Gynex, Inc. and an NIH grant HD20498).

425.7 DIFFERENT GABA RECEPTOR TYPES EXERT OPPOSING EFFECTS ON GONADOTROPIN SECRETION: IN VITRO AND IN VIVO EVIDENCE. C. Masotto* and A. Negro-Vilar (SPON: J. D. Mann). Reprod. Neuroendo. Sect., Lab. Reprod. Dev. Tox., NIEHS, NIH, Research Triangle Park, NC 27709.

In the last few years, it has become evident that the neurotransmitter γ-aminobutyric acid (GABA) may have an important role in the control of gonadotropin secretion. However the precise role played by GABA and its analogs in the control of the gonadotropins has not yet been fully clarified. The main purpose of this study was to investigate the involvement of different GABA receptors on LHRH release and LH secretion. In order to explore this issue we performed both in vitro and in vivo experiments using adult male intact Sprague-Dawley rats. In the in vitro experiments, a hypothalamic fragment consisting of the intact arcuate nucleus-median eminence region (ARC-ME) was dissected and incubated in a Krebs buffer. LHRH release was evaluated during a 30 min incubation period. Incubation of the tissue with different concentrations of muscimol (10^{-6} to 10^{-4}M), a specific GABA-A receptor agonist, increased the release of LHRH from the ARC-ME fragment. Baclofen (10^{-4}M), a GABA-B receptor agonist, did not affect basal LHRH release. The effects of muscimol were completely blocked by the specific GABA receptor antagonist, bicuculline. In the in vivo experiments, the animals were implanted with a silastic cannula in the right jugular vein in order to collect frequent blood samples (every 10 min up to 80 min) before and after drug administration. In these experiments, the intravenous (iv) administration of aminooxyacetic acid (AOAA, 25 mg/kg BW), an inhibitor of GABA catabolism which has been shown to increase brain GABA levels, completely prevented the rise in LH induced by naloxone (5 mg/kg BW, iv). Baclofen (6 mg/kg BW, iv) given 20 min before naloxone prevented the naloxone-stimulated LH secretion. On the other hand, muscimol (2 mg/kg BW, iv) does not affect the rise in LH induced by naloxone. These effects were duplicated in vitro, since baclofen blocked the naloxone-stimulated LHRH release, while muscimol had an additive effect with naloxone to increase LHRH release from the ARC-ME. The results of this study suggest that the GABAergic system exerts both an inhibitory and stimulatory effect on LH secretion. The data indicate that the GABA-B receptors are responsible for the inhibitory effect observed in vitro and in vivo, particularly during situations of stimulated LHRH release and increased LH secretion. On the other hand, GABA-A receptors in the ARC-ME region mediate the stimulatory effect of GABA on LHRH release. The finding that different GABA receptor types mediate opposite effects on LHRH and LH may help to clarify the exact role of this neurotransmitter in the regulation of gonadotropin secretion.

425.8 DEVELOPMENT OF SPECIFIC ANTISERA FOR QUANTITATION OF INHIBIN IN BIOLOGICAL FLUIDS AND FOR PASSIVE IMMUNONEUTRALIZATION. M. D. Culler* and A. Negro-Vilar. Reprod. Neuroendo. Sect., Lab. Reprod. Dev. Tox., NIEHS, NIH, P.O. Box 12233, Research Triangle Park, NC, 27709

Recently, the cDNAs which encode for gonadal inhibin in the porcine, bovine and human were isolated and their corresponding peptide sequences deduced. Two forms of inhibin, A and B, were discovered for each species. Both inhibins are composed of two subunits: an alpha subunit that is common to both forms joined by multiple disulphide linkages to one of two beta subunits, β_A or β_B. Since the α-subunit is common to both forms of inhibin, we used a fragment of the N-terminal region of the human α-inhibin subunit ($h\alpha IN_{1-32}$), conjugated to human serum albumin, as antigen to generate two specific antisera in sheep (MC-4 and 5). Using ^{125}I-labeled $h\alpha IN_{1-32}$ as tracer, the MC-4 and 5 sera produce 30% specific binding with final dilutions of 1:48,000 and 1:192,000, respectively. Concentration dependent inhibition of tracer binding can be achieved with unlabeled $h\alpha IN_{1-32}$ over a range of from 1.35 - 2.70 to 1380 fmoles. Concentration dependent inhibition of tracer can also be achieved using serial dilutions of porcine and human ovarian follicular fluid, media taken from cultures of rat testicular Sertoli cells (courtesy of Dr. A. Kierszenbaum, UNC) and intact rat plasma. These results suggest that the antisera can recognize the α-inhibin subunit of at least these three species and probably the entire inhibin molecule since a previous study found no free α-subunits in bovine follicular fluid (Miyamoto et al., BBRC 136:1103, 1986). In order to initiate studies using the anti-inhibin sera to passively immunoneutralize endogenous inhibin, intact adult male rats, under light Metofane anesthesia, were injected with anti-inhibin serum MC-5 or normal sheep serum into the jugular vein immediately following the withdrawal of a 0.5 ml blood sample. Subsequent blood samples were collected at 2, 6, 12, 24 or 48 hrs after injection. Plasma FSH and LH were determined by RIA, using NIADDK reagents, after it was determined that the ovine anti-inhibin serum did not interfere with the assays. A bolus, 0.5 ml injection of the MC-5 antisera was sufficient to produce a significant, though transient, elevation of plasma FSH after 6 hrs as compared with rats injected with normal sheep serum. Plasma LH was not affected by the injection of antiserum. These results suggest that inhibin may play a role in the normal maintenance of plasma FSH in the male. The return of plasma FSH to normal levels by 24 hrs after antiserum injection suggests the involvement of compensatory mechanisms. The ability to immunoneutralize endogenous inhibin and to measure inhibin secretion in biological fluids will provide powerful tools for the study of inhibin physiology.

425.9 IN VIVO LHRH AND 5-HIAA LEVELS AS MEASURED WITH PUSH-PULL PERFUSION OF THE ANTERIOR PITUITARY (AP) OF OVARIECTOMIZED FEMALE RATS WITH OR WITHOUT AN ESTROGEN IMPLANT. O-K Park* and V. D. Ramirez (SPON: L. Barr). Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

Herein, we report in vivo LHRH and 5-HIAA levels as measured with push-pull perfusion of the AP of ovariectomized (OVX) rats under the following conditions: 1) short term OVX (8-10 d), 2) long term OVX(~60 d) and 3) long term OVX with an estrogen implant. Rats (240-260 g) were anesthetized with Ketamine-Acepromazine (1:10) for surgery. Bilateral ovariectomy was performed and a push-pull cannula was implanted aimed at the AP. After surgery, rats were kept in an individual cage at 22°C under a 14L:10D photoperiod (light on at 0500 h). Pituitaries were perfused at a flow rate of 11-12 µl/min with a modified KRP medium containing 0.1 mM Bacitracin. Perfusate samples were collected on ice every 10 min, from 1100 to 1800 h. While being perfused, rats showed normal behaviors such as sleeping and grooming. An aliquot (20 µl) of each sample was immediately injected to an HPLC-EC for the analysis of 5-HIAA while the remaining of the sample was acidified and kept frozen until LHRH RIA. The individual data for LHRH were analyzed with PC-PULSAR. On completion of the first perfusion, rats were returned to the animal facility room. At the end of the second perfusion which was performed about 60 days after OVX, rats were anesthetized with ether and implanted with an 11 mm silastic capsule containing 235 µg/ml estradiol. About 43 h later, rats were perfused in the same manner and sacrificed for the examination of PPC placement.

No significant changes in LHRH levels were observed among the experimental groups. Mean LHRH values in the groups of short term OVX (n=4), long term OVX (n=3), and long term OVX with an estradiol implant (n=3) were 0.86 ± 0.19, 0.97 ± 0.14 and 1.08 ± 0.28 pg/10 min, respectively. The mean frequency and amplitude of LHRH pulses were similar among the groups (1 pulse every 70 ± 9.8 min and 1.13 ± 0.12 pg, respectively). 5-HIAA was observed to undergo remarkable changes as a function of these conditions. Long term OVX showed significantly elevated 5-HIAA levels as compared to those obtained from short term OVX (60.0 ± 41.0 vs 5.24 ± 4.24 pg/min). These elevated levels of 5-HIAA were reduced to 4.71 ± 4.59 pg/min in the group of long term OVX rats treated with estrogen.

These results show that 1) push-pull perfusion of the pituitary can be used to analyze changes in neuropeptides and neurotransmitters simultaneously, 2) LHRH activity as measured with push-pull perfusion of the pituitary did not change as function of OVX nor was it modified by estradiol, 3) however, 5-HIAA levels were elevated in long term OVX rats and these levels were reduced to those of short term animals by estradiol.

425.10 PULSATILE GnRH RELEASE FROM THE HUMAN MEDIOBASAL HYPOTHALAMUS (MBH): IN VITRO SUPPRESSION BY MORPHINE. D.D. Rasmussen, M. Gambacciani*, J.H. Liu*, W.H. Swartz*, V.S. Tueros*, S.S.C. Yen*. Dept. of Reproductive Medicine, UCSD, La Jolla, CA 92093.

Neither hypothalamic pulsatile GnRH secretion nor the site of the putative GnRH pulse generating mechanism have been demonstrated in the human. Accordingly, an in vitro perifusion system was used to investigate pulsatile GnRH release from human fetal (21-23 wk gestation) and adult hypothalami (obtained at autopsy within 5.5 h of fetal death and 12 h of adult death). After a 3 h stabilization period each of 7 fetal MBHs released GnRH (RIA, assayed in triplicate) in a strikingly pulsatile pattern with a mean (±SE) release rate of 9.9±1.5 pg/10 min. PULSAR pulse detection computer analysis (Merriam and Wachter, Am J Physiol 243:E310) of the GnRH release pattern during fetal MBH perifusions with medium alone throughout the 13 h experimental period (n=3) revealed that significant GnRH pulses with amplitudes of 5.1±0.9 pg/10 min occurred at intervals of 60.6±4.3 min. Addition of 10 µM each of the membrane calcium channel blockers nifedipine and verapamil to the medium midway through the perifusion of another of the fetal MBHs dramatically suppressed pulsatile GnRH release from 1 pulse/43.8±6.5 min (amplitude: 10.0±1.0 pg/10 min) to 1 pulse/400 min (amplitude: 3.59 pg/10 min). Addition of 1 µM morphine to the medium midway through the perifusions of 3 of the fetal MBHs suppressed the frequency of pulsatile GnRH secretion from 1 pulse/135±72 min (amplitude: 3.8±0.7 pg/10 min) to 1 pulse/220±72 min (amplitude: 3.5±0.6 pg/10 min), whereas subsequent addition of 1 µM naloxone restored the frequency to 1 pulse/62.2±7.8 min (amplitude: 3.8±0.3 pg/10 min). An adult MBH also released GnRH in a pulsatile pattern throughout the 13 h experimental period, with an overall release rate of 9.8 pg/10 min, pulse interval of 85±4.3 min, and pulse amplitude of 3.7±0.4 pg/10 min. In addition, each half of an adult MBH, bisected longitudinally at the median eminence, also released GnRH in a pulsatile pattern, with overall release rates of 7.6±3.5 pg/10 min, pulse intervals of 78.7±18.7 min, and pulse amplitudes of 3.9±0.5 pg/10 min. These results demonstrate for the first time that the mechanism mediating pulsatile GnRH release is contained entirely within the human MBH, that the frequency of this pulsatile GnRH release is comparable to that of circulating LH pulses in vivo, and that opiate suppression of this calcium dependent pulsatile GnRH release can also be mediated directly within the MBH.

425.11 BIOLOGICAL PROPERTIES OF LUTEINIZING HORMONE RELEASE INHIBITING FACTOR. Jong-Chin Hwan, Jon E. Levine and Marc E. Freeman*. Department of Biological Science, Florida State University, Tallahassee 32306 and Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60201.

We have recently reported that the rat hypothalamus contains an inhibitor of LHRH-stimulated LH release (Hwan and Freeman, Endocrinology 120:483, 1987). This inhibitor, designated luteinizing hormone release inhibiting factor (LHRIF), depressed LHRH-stimulated LH release in proestrous rats as well as in monolayer cultures of enzymatically dissociated anterior pituitary cells (APC). The factor also depressed LH release induced by D-Ala^6 des Gly^{10} LHRH suggesting that LHRIF is not merely a peptidase. LHRIF had neither an inhibitory nor stimulatory effect on basal LH or prolactin secretion from APC. Though devoid of biological activity by itself, LHRIF cross reacted with LHRH in a well characterized radioimmunoassay. Though a potent inhibitor of LHRH-stimulated LH release, LHRIF did not inhibit potassium- or prostaglandin D_2-stimulated LH release. These data suggest, indirectly, that LHRIF may act through recognition of the LHRH receptor. LHRIF was localized in subcellular fractions of medial basal hypothalami obtained from ovariectomized rats. Tissue was homogenized in 0.32 M sucrose and pelleted to yield synaptosomes and crude mitochondria. This fraction, designated P_2, was separated into 6 subfractions in a discontinuous sucrose gradient. Fractions were characterized by immunoblotting on nitrocellulose using a polyclonal antiserum to LHRIF. Only fraction P_2-3 showed strong reaction with this antiserum. This same fraction, containing myelin fragments, syptosomes, and secretory vesicles, inhibited LHRH-stimulated LH release from APC in a dose-dependent manner. As before, this fraction did not affect basal LH or prolactin secretion. Fraction P_2-5 stimulated LH release. All other fractions were inactive. These data suggest that LHRIF is a physiologically significant neuronal product involved in the control of LH secretion from the pituitary gland. Supported by the Mellon Foundation.

425.12 N-METHYL-DL-ASPARTATE LESIONS OF THE ARCUATE NUCLEUS DO NOT DISRUPT THE PULSATILE SECRETION OF LUTEINIZING HORMONE IN MALE RATS. C.L. Sisk, A.A. Nunez, and M.M. Thebert*. Neuroscience Program and Dept. of Psychology, Michigan State University, East Lansing, MI 48824

Several lines of evidence suggest that the arcuate nucleus (ARC) may generate neural signals for the pulsatile release of LHRH. (Neuroendo. 35: 63; Ann. Rev. Phys. 44: 583). Electrolytic lesions of the ARC/median eminence (ME) of male rats either reduce the frequency and amplitude of LH pulses or abolish LH secretion (Soc. Neurosci. Abst. 12: 1414). However, the effects of damage to ARC neurons could not be dissociated from those due to destruction of LHRH fibers projecting to ME. This study was designed to distinguish between these two possibilities by making axon-sparing chemical lesions in ARC. All surgeries were performed on anesthetized animals. 60-day-old male rats were castrated; one week later, 2 25µl injections of 0.3M NMA in artificial CSF or sham injections of CSF were aimed bilaterally at ARC. After 10 days, blood samples were obtained at 5 min intervals for 4 hr via a jugular cannula. Rats were then perfused with saline and Zamboni's fixative. Alternate coronal sections through the hypothalamus were either stained with cresyl violet or processed for LHRH immunocytochemistry using the ABC method and Benoit LR-1 as primary antiserum. Plasma concentrations of LH were determined by RIA; hormone pulses were quantified by the PULSAR program. Based on histological results, rats receiving NMA injections were classified as having either minimal or extensive damage to ARC. In the latter group, the vast majority of ARC neurons was destroyed, as well as most neurons in the ventromedial nucleus. In this respect, these lesions were similar to our earlier electrolytic lesions. However, the integrity of ME and the distribution of LHRH fibers within it appeared normal after NMA injections. Regardless of the degree of damage incurred to ARC, rats receiving NMA lesions secreted LH pulses with frequencies similar to those of sham-injected controls (about 3 pulses/hr). These results suggest that the effects of electrolytic ARC lesions on LH secretion were due to destruction of fibers of passage and not to destruction of ARC neurons per se. They further suggest that ARC plays little or no role in the generation of periodic neural signals for the release of LHRH in the castrated male rat. Supported by HD-21588 and MH-37877.

425.13 OVARIAN INDEPENDENT DEVELOPMENT OF OPIOID REGULATION OF LUTEINIZING HORMONE (LH) SECRETION IN THE PREPUBERAL PIG. C. R. Barb*, G. B. Rampacek*, and R. R. Kraeling* (SPON:C. J. Karwoski). USDA-ARS R. B. Russell Research Center, Athens, GA 30613 and University of Georgia, Athens, GA 30602.

Previous work indicated that naloxone (NAL), an opioid antagonist, increased serum LH concentrations only during the luteal phase of the estrous cycle in the sexually mature pig (Barb et al., Biol. Reprod. 35:1162, 1986). However, in the intact or ovariectomized (OVX) progesterone (P4) treated prepuberal pig, NAL failed to alter LH secretion (Barb et al., Biol. Reprod. 34:Suppl. 1. 1986). The following study was conducted to determine if development of endogenous opioid peptide (EOP) modulation of LH secretion in the prepuberal pig is dependent on estradiol-17β (E) priming or is a brain maturation process independent of the ovary. Fifteen OVX pigs, 280 days of age and 132 ± 2.3 kg body weight (BW), were used. Ten pigs were OVX at 160 days of age which was prior to puberty and were classified as chronologically mature (CM) based on their age at the time of treatment. The remaining five pigs had displayed two or more estrous cycles prior to OVX (puberty = 197 ± 10 days), and were classified as sexually mature (SM). In addition, 5 pigs were OVX at 160 days of age and 77.9 ± 3.2 kg BW and were prepuberal (P) at the time of the experiment at 170 days of age. All pigs were injected with P4 (0.85 mg/kg BW) twice daily for 10 days. Four days prior to the start of P4 treatment, five CM pigs were injected with E (1 μ/kg BW) resulting in the following groups: SMP4 (n = 5), CMP4 (n = 5), CMEP4 (n = 5) and PP4 (n = 5). All pigs received 1 mg/kg BW of NAL in saline iv on the last day of treatment. Blood was collected via jugular cannula every 15 min for 4 h before and 2 h after NAL treatment. All pigs received GnRH (0.25 μg/kg BW) in saline iv and blood collected an additional h. NAL treatment resulted in increased ($P<0.05$) serum LH concentrations in the SMP4, CMP4 and CMEP4 pigs. However, NAL treatment failed to alter serum LH concentration in the PP4 pigs. Serum LH concentrations averaged 0.40 ± 0.04, 0.51 ± 0.04 and 0.28 ± .03 ng/ml prior to NAL and increased ($P<0.05$) to 2.03 ± 0.19, 1.19 ± 0.12 and 1.02 ± 0.19 ng/ml during the first h after NAL for the SMP4, CMP4 and CMEP4 pigs, respectively. Serum LH concentration averaged 0.44 ± 0.07 ng/ml prior to and 0.26 ± 0.06 ng/ml after NAL in PP4 pigs. Serum LH response to GnRH was greater ($P<0.05$) in PP4 pigs (5.75 ± 0.67 ng/ml) than in SMP4 (2.33 ± 0.22 ng/ml), CMP4 (2.61 ± 0.21 ng/ml) and CMEP4 (2.60 ± 0.22 ng/ml) pigs. We suggest that development of EOP modulation of LH secretion is a brain maturational process independent of the ovary in the prepuberal pig.

425.14 BINDING OF RADIOIODINATED FSH-RELEASING PROTEIN (FRP) TO PITUITARY MEMBRANES AND LEUKEMIA CELLS. Carolyn A. Campen* and Wylie Vale (SPON: R. Evans). The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037.

Recently, several proteins regulating cell secretion, division or differentiation were found to share significant sequence homology. These proteins include Transforming Growth Factor β (TGF-β), inhibin, FSH-Releasing Protein (FRP), Mullerian Inhibiting Substance (MIS), and fly decapentaplegic gene complex (DPP-C). Inhibin is a heterodimeric protein (αβ) and FRP is a homodimeric protein composed of inhibin β subunits (ββ), both initially purified from gonad extracts. The first biological activity associated with FRP was the ability to stimulate the biosynthesis and release of FSH from rat pituitary cells in vitro. Because receptor binding is assumed to be the first step in FRP action, it was of interest to characterize the binding of FRP to pituitary cells.

FRP was radioiodinated using chloramine T and purified by HPLC. Using a crude rat pituitary membrane preparation, only 3-5% of the total counts added bound, and approximately 50% of these counts could be displaced using a preparation of native FRP. Because the heterogeneity of cell types within the pituitary may contribute to the low amount of binding, it was of interest to look for other sources of receptor for initial characterization.

Recently, Eto and coworkers described the isolation and characterization of Erythroid Differentiation Factor (EDF) which stimulates the differentiation of mouse erythroleukemia cells (MEL cells). EDF is also a homodimeric protein that appears to be identical to FRP based on the identity of the N-terminal sequence of both proteins. Because EDF/FRP is biologically active on MEL cells, the binding of ^{125}I-FRP on MEL cells was explored. Significantly higher specific binding was observed using MEL cells as compared to pituitary membranes. Incubation of 5 x 10^6 MEL cells with ^{125}I-FRP (~ 0.15 nM) resulted in the binding of 10-15% of the total counts added, about 70% of which could be displaced using a preparation of native FRP as unlabeled competitor. The estimated K_d of the MEL cell FRP binding site is 0.3 - 0.4 nM. Unrelated proteins, FSH and EGF, did not cause displacement of the ^{125}I-FRP from MEL cells. Other methods of binding characterization were also explored. ^{125}I-FRP also binds to monocyte leukemia cells suggesting that FRP binding may be common to hematopoietic cell types in addition to specific cell types in the pituitary. Further characterization of the pituitary and the MEL cell FRP binding sites may illuminate the physiological relevance of FRP.

425.15 MONOSODIUM GLUTAMATE TREATMENT ALTERS ANESTHESIA AND BETA-ENDORPHIN STIMULATED PROLACTIN SECRETION IN FEMALE RATS: ASSOCIATION WITH TYROSINE HYDROXYLASE IMMUNOCYTOCHEMISTRY. Richard W. Clough, Susan W. Carlson*, Carol J. Phelps, Paul E. Aravich* and Celia D. Sladek Dept. Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642

Administration of MSG to neonatal rats produces lesions in the arcuate nucleus (ARC) of the hypothalamus including the tuberoinfundibular dopamine cells (TIDA) which tonically inhibit prolactin secretion. The present study examined 1) the extent of damage to hypothalamic tyrosine hydroxylase (TH) and vasoactive intestinal polypeptide (VIP, a putative prolactin releasing factor) containing neurons and 2) anesthesia and intracerebroventricular beta-endorphin (B-end) stimulated prolactin secretion in MSG-treated compared to saline-treated control rats. MSG (8 mg/g body weight, sc) or sodium chloride (sodium equimolar to that in MSG) was administered to rats on day 4 after birth. At 2 months of age, rats were anesthestized with chlorohydrate-pentobarbitol (.3cc/100 g body weight) and blood was withdrawn by jugular venipuncture at 20 and 60 minutes following anesthesia. After the 60 min sampling, each rat was placed into a stereotaxic apparatus and prepared for an intracerebroventricular infusion of B-end (5 ug/5 ul sterile H_2O). B-end was infused over a 5 min interval (1 ul/min) starting 10 minutes after the 60 min sample. Blood was again withdrawn by jugular venipuncture at 20 min after endorphin and plasma was prepared for radioimmunoassay of prolactin (anti-prol. S-9, RP#3, NIAMDDK). Each bleeding was followed by volume replacement with heparinized saline. After the terminal bleeding, rats were perfused transcardially with 4% paraformaldehyde and brains were sectioned for TH and VIP immunocytochemistry. MSG-treated rats had a significantly attenuated prolactin response to the stress of anesthesia (bleeding 1) compared to the saline-treated controls. The prolactin levels in saline and MSG-treated rats were similar at 60 min post-anestheisa (bleeding 2) which represented a return to baseline by prolactin levels in the control rats. MSG- and saline-treated rats exhibited an increase in plasma prolactin following intracerebroventricular B-end; however, the amplitude of this response was markedly and statistically attenuated in the MSG treated animals (bleeding 3). An obvious, although not total, loss of TH immunopositive cells in the ARC region of the hypothalamus was observed while there was no apparent reduction of TH staining in the incertohypothalamic neuronal groups (A11 and A13). VIP immunocytochemistry in the suprachiasmatic region appeared to be similar in control and MSG-treated rats. VIP staining in the ARC region was not found to be different between MSG- and Saline-treated rats. The observed loss of ARC TIDA cells following MSG treatment suggests that attenuated prolactin release in response to stress and B-end in MSG-treated rats, occurs as result of loss of dopamine in the ARC region of the hypothalamus. This suggests that the prolactin increase in the control rats to both stress and B-end is mediated by an inhibition of dopamine release from the TIDA system and not through the putative prolactin releasing effects of VIP. Supported by PHS grant 1 F32-MH09136.

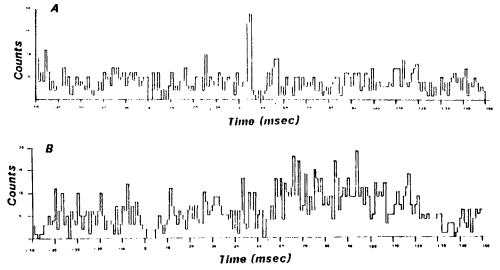
425.16 THE ROLE OF INCREASED CORTISOL SECRETION IN THE SUPPRESSION OF GnRH PULSE GENERATOR ACTIVITY IN MONKEYS PLACED ON A REGIMEN OF REDUCED FOOD INTAKE. J.L. Cameron. Departments of Psychiatry and Behavioral Neuroscience and the Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213.

Previous experiments have demonstrated that 50-75% of adult male monkeys placed on a reduced food intake (RFI) regimen (e.g., consuming about one quarter of their normal food intake for approximately 3 weeks) have a suppression of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion resulting from reduced gonadotropin releasing hormone (GnRH) secretion (Dubey et al., Endocrinology 118: 518-525, 1986). The decrease in gonadotropin secretion during RFI is accompanied by an increase in mean circulating levels of cortisol. To examine this relationship more closely, 6 castrate adult male rhesus monkeys were placed on the RFI regimen and blood samples were collected by femoral venipuncture every 2-3 days for measurement of LH, FSH and cortisol. Results showed that circulating cortisol levels rose sharply (to levels between 80-120 μg/dl), coincident with the decline in circulating LH and FSH secretion, in monkeys which had a decrease in gonadotropin secretion during RFI. In contrast, no abrupt increase in circulating cortisol was detected in monkeys which did not show a decrease in circulating gonadotropin levels during RFI. This correlation suggested that a rise in circulating cortisol may play a role in suppressing gonadotropin secretion during RFI. To test this hypothesis, 5 additional monkeys were given daily injections (i.m.) of hydrocortisone acetate (HCA; kindly donated by the Upjohn Co.) in a regimen designed to mimic the rise in cortisol found during RFI (i.e., increasing doses from 5-20 mg/kg over a 3-4 week period). Blood samples were collected 3-4 times per week by femoral venipuncture. All 5 monkeys showed a pattern of rising cortisol levels similar to the rise in serum cortisol found in monkeys whose gonadotropin levels declined during RFI. However, neither serum LH or FSH decreased in the 5 monkeys which received HCA. These results indicate that the rise in circulating cortisol is not responsible for the suppression of GnRH neuronal activity during RFI.

Supported by NIH grants HD20789 and HD20887.

426.1 THE EFFECTS OF SURAL NERVE STIMULATION ON THE FIRING OF SOLEUS MOTOR UNITS IN MAN. C.G. Kukulka, D.A. Brown*. Physical Therapy Labs. The University of Iowa College of Medicine, Iowa City, IA 52242.

Cutaneous afferent effects on motoneuron excitability have been studied primarily using noxious skin stimulation to evoke flexion reflexes. Much less is known about the effects of nonnoxious cutaneous stimulation. The purpose of this study was to evaluate the effects of nonnoxious levels of electrical stimulation of the sural nerve on the discharging of human soleus motor units. Bipolar fine wire electrodes were used to record motor units intramuscularly from soleus muscle. Electrical stimuli were delivered to the sural nerve inferior to the lateral malleolus. Two stimulation paradigms were used. One consisted of single, 0.1 msec pulses at 3 pulses/s. The second consisted of 500/s trains of 0.1 msec pulses of durations varying from 5 to 10 msec delivered at 3 trains/s. The effect of stimulation was evaluated by constructing peristimulus time histograms. Two general findings were observed. The first, produced by single pulse stimulation, consisted of short latency excitation-inhibition-excitation, initiated at latencies of 42-44 msec and lasting 10-14 msec.(Fig. A). The inhibitory effect has been consistently seen in all 3 subjects tested to date. The excitatory effects are more labile and appear highly sensitive to the intensity of stimulation. The second finding (Fig. B), produced by pulse train stimulation, consisted of long latency excitation - inhibition of much longer duration. Excitation was observed at latencies of 54-56 msec, and lasted for durations of 64-66 msec. Inhibition was observed at 120-124 msec and lasted for 26-30 msec. These findings suggest that the two different types of stimulation may activate different spinal and/or supraspinal pathways involved in nonnoxious cutaneous reflexes.



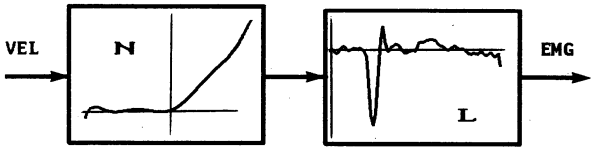
426.2 **NONLINEAR IDENTIFICATION OF A HAMMERSTEIN MODEL OF HUMAN TRICEPS SURAE STRETCH REFLEXES. PART I: METHOD.** Ian Hunter & Rob Kearney. Biomedical Engineering Unit, McGill University, Montreal, Canada.

The dynamic relation between the angular velocity of a limb (input) and the resulting mean rectified electromyogram (EMG) (output), sometimes termed the stretch reflex, has been previously modeled as a half-wave rectifier (static nonlinear, **N**, element) followed by a linear filter (dynamic linear, **L**, element). Such a nonlinear dynamic model is known as a Hammerstein system (see figure below). Until recently system identification techniques appropriate for Hammerstein systems have required Gaussian-white inputs (i.e. Gaussian first-order amplitude probability density function and constant first-order power spectral density function) and a monotonically increasing (or decreasing) **N** element. These techniques have not been used because Gaussian-white angular displacement perturbations are difficult to arrange in practice.

Hunter and Korenberg (1986) have recently presented a Hammerstein identification technique which does not require either Gaussian-white inputs or monotonic **N** elements. Furthermore, the technique is nonparametric so a priori assumptions about the form of the nonlinearity (**N**) and order of the dynamic linearity (**L**) are not required.

The Hammerstein nonlinear system identification method involves an iterative approach in which the static nonlinear element, **L**, is represented by a polynomial (whose order is determined by a model order selection criterion) and the dynamic linear element, **L**, is estimated using either singular value decomposition or Toeplitz matrix inversion techniques. We have found that the technique is robust in the presence of large amounts of noise corrupting the EMG signal.

Using large, non-Gaussian, non-white stochastic angular displacement perturbations of the human foot about a fixed mean ankle joint angle, we have successfully used the technique to obtain nonparametric estimates of the **N** and **L** elements of the Hammerstein representation of stretch reflex dynamics (see Part II). Representative **N** and **L** elements determined by the method are shown in the Figure. (Supported by NSERC and MRC)



426.3 **NONLINEAR IDENTIFICATION OF A HAMMERSTEIN MODEL OF HUMAN TRICEPS SURAE STRETCH REFLEXES. PART II: RESULTS.**
R.E. Kearney, I.W. Hunter, & P.L. Weiss, Biomedical Engineering Unit, McGill University, Montréal, Québec, Canada

Stretch reflexes in the human triceps surae may be described in terms of the dynamic relation between joint velocity and the resulting EMG activity. This relation is very nonlinear; we have previously modeled it as a Hammerstein system consisting of a static nonlinearity cascaded with linear dynamics [2]. The form of the static nonlinearity was found by trial and error to resemble a unidirectional rate sensitive element or half-wave rectifier. This model gave adequate descriptions of reflex behavior for particular conditions but was clearly incomplete since estimates for the linear dynamic component changed with displacement amplitude and joint position. One explanation for this behavior is that the static nonlinearity is more complex than a unidirectional rate sensitive element. We undertook to explore this possibility using a new nonlinear identification method which produces direct estimates of both the static nonlinear and the dynamic linear components of Hammerstein systems [1].

Subjects were trained to develop constant mean levels of ankle torque while subjected to perturbations of ankle angular position. The perturbation was a stochastic signal containing significant power to 50 Hz; the peak-to-peak amplitude of the perturbation was modulated from 0.0 - 0.25 rad in a ramp waveform with a period of 16 s. Ankle position, torque and EMG activity were sampled at 500 Hz for 80 s. Estimates of the static nonlinear and dynamic linear components of a Hammerstein model were then obtained using the iterative procedure described in [1].

The static nonlinearity and linear dynamics, represented by the impulse response function, were found to have characteristic forms which were similar from trial to trial and subject to subject. In agreement with our previous findings the linear impulse response functions were dominated by a large excitatory peak at about 40 ms. The static nonlinearity displayed a definite direction dependent rate sensitivity; positive velocities (stretch of triceps surae) were passed with a much higher gain than negative velocities. The nonlinear models identified in this work are in general agreement with our previous findings. However, the new models predicted EMG responses more accurately than our previous models. Experiments are in progress to determine whether this results in models having a wider range of applicability.

Supported by the Canadian Medical Research Council and the Natural Sciences and Engineering Research Council of Canada.
1. Hunter & Kearney, Neuroscience Abstracts, (1987).
2. Kearney & Hunter, Exp. Brain Res., (1983) 51:117-127.

426.4 EMG RESPONSES AT VARYING STRETCH AMPLITUDES IN ANTERIOR TIBIAL MUSCLE AT DIFFERENT LEVELS OF VOLUNTARY CONTRACTION IN HUMANS. E. Toft*, T. Sinkjaer* and S. Andreassen* (SPON: R.L. Watts). Institute of Electronic Systems, Aalborg University, Strandvejen 19, DK-9000 Aalborg, Denmark.

When an active flexed joint is abruptly extended, there is an increase in the EMG activity of the flexor muscles and the torque around the joint increases. The objective of this study was to investigate the significance of the different parts of the EMG reflex in the anterior tibial muscle at varying voluntary contraction (background torque) and stretch amplitudes.

During voluntary contraction of the ankle flexors, the ankle was periodically plantar flexed by a servo-controlled motor. The subjects were asked to maintain a constant level of background torque varying from 0 to 80% of maximum voluntary contraction (MVC) during which 50 sweeps were done. Stretch amplitude was varied between 1, 2, 4 and 7 degrees. Maximum stretch velocity was kept constant at 100 degrees/s and risetime proportional to stretch amplitude. Averaged EMG responses showed three distinct peaks labeled M1, M2 and M3, with latencies ranging from 57-62 ms, 79-93 ms and 110-123 ms respectively. Latencies decreased slightly for M1 and M2, but more pronounced for M3 with increasing background torque. The decreases were linear.

For increasing stretch amplitude, the peak latencies decreased for M3 at stretch of 4 and 7 degrees, but was unaffected for M1 and M2. With increasing background torque, the amplitude rose. The rise was slight for M1 and much more pronounced for M2 and M3. For the highest background torques, M2 and M3 were the dominating peaks. For increasing stretch amplitudes, the peak of M1 was unaltered or increased slightly, whereas the amplitudes of M2 and M3 increased markedly. At low background torques, the M2 and M3 were low or absent at 1 and 2 degree stretches, but comparable to M1 at 4 and 7 degree stretches.

Simultaneous recordings revealed torque increments almost proportional to the stretch amplitude. This increase must have been accomplished by the increase in M2 and M3 and emphasizes the mechanical significance of M2 and M3.

The occurrence of M2 and M3 is a matter of much debate. In this study, the M2 and M3 increased with increased stretch amplitude whereas M1 was unchanged or slightly increased. M1 is known to be a monosynaptic reflex elicited from the velocity sensitive muscle spindles. In this experiment the maximum stretch velocity was kept constant and the rise time proportional to stretch. These results therefore seem to contradict the theory that M1, M2 and M3 should be elicited from consecutive bursts in Ia afferents (Hagbarth, K.E., Hagglund, J.V., Wallen, E.U. & Young, R.R., J. Physiol., 312, 1981).

426.5 INCREASED INHIBITORY EFFECTS ON CLOSE SYNERGISTS DURING MUSCLE FATIGUE. L. Hayward, D. Breitbach and W.Z. Rymer . Dept. of Physiology, Northwestern Univ., Chicago, IL 60611.

Investigations of human motor unit discharge during sustained maximum voluntary contractions have demonstrated that motor unit firing rate declines during muscle fatigue. This fatigue-induced change is presumed to help optimize force generation in the fatiguing muscle. Although some of this rate modulation may be attributed to central changes (Kernell & Monster, Exp. Br. Res., 46:191,1982), recent evidence supports the role of a peripheral feedback mechanism from the fatiguing muscle (Bigland-Ritchie, et al., J. Physiol.,379:451,1986). In this study we examined some characteristics of this fatigue related feedback mechanism by comparing the influence of MG fatigue on force production of a non-fatigued close synergist, SOL, in decerebrate cats.

MG was selectively fatigued by muscle-nerve stimulation at 1.3 x Group I threshold (xT) and the influence of fatigue-related afferent feedback was measured in the non-fatigued soleus muscle. Force,EMG and length were recorded from MG and SOL during either 3 sec (25-50Hz) stimulation of MG muscle-nerve or ramp stretches (3-8mm) of MG. Initial SOL force was established by crossed extension reflex. Before fatigue, a decline in SOL force was recorded during isometric or stretch stimulation of MG. This reflexly induced decrement in SOL force increased as MG force production increased. During fatigue (defined as MG force generation at 30% original value) this decrement in SOL force increased. The average SOL decrement during fatigue of MG was significantly greater ($p>0.05$) after fatigue. After 30-50 minutes rest SOL force decrement during MG stimulation returned to control values.

This reflex inhibition may be mediated by afferents stimulated by mechanical, thermal or metabolic changes during fatigue. To identify the afferents responsible for the reflex inhibition of the SOL force during MG stimulation and fatigue, the MG muscle-nerve was sectioned and stimulated proximally. With stimulus intensities in Group I threshold range (1.1-1.5xT) low frequency stimulation rates (20-50Hz) produced little or no effect on SOL force generation. However, higher stimulation frequencies (100-200 Hz) consistently produced small decrements in SOL force, suggesting a possible contribution from Ib afferents. Greater increases in SOL decrement were produced only at higher stimulus intensities, 20xT (50-200Hz), within the range of Group III and IV afferents. Since these afferents are responsive to thermal and chemical stimuli it is possible that their discharge increased during muscle fatigue, contributing to the increase in SOL inhibition reported here. This study supports the proposition that small fiber afferent discharge elicited during muscle fatigue inhibits regional MN pools.

426.6 PROLONGED MUSCLE STRETCH CAN MODIFY REFLEX AND VOLUNTARY MUSCLE ACTIVATIONS IN SPASTIC CEREBRAL PALSY. F. Malouin, F. Tremblay*, C.L. Richards and F. Dumas*. Physiotherapy Department and Neurobiology Laboratory, Faculty of Medicine, Laval University, Quebec, Canada G1K 7P4.

We studied the short-term effects of a single session of prolonged muscle stretch (PMS) of the plantarflexors on reflex and voluntary muscle activations in 21 children (3 to 14 yrs) with spastic cerebral palsy (2 tetraparetics, 13 diplegics and 6 hemiplegics) assigned to an experimental (exp) and a control (ctl) group. Neuromuscular responses to passive ankle movements were measured with the child sitting (hip and knees flexed), with the right foot attached to a footplate. The ankle axis was aligned with the rotational axis of a KIN-COM dynamometer which imposed passive dorsiflexion (DF) and plantarflexion (PF) at 30, 60 and 120°/s. Each trial consisted of 5 repetitions from +35° PF to -5° of DF with a 1s pause separating each change of direction. Subjects then performed 3 maximal voluntary isometric PF and DF contractions with the ankle at +10° PF. During both the passive movements and isometric contractions, the electromyographic (EMG) activity was picked up with surface electrodes from the triceps surae (TS) and tibialis anterior (TA) muscles. The myosignals were amplified, and viewed as raw EMG prior to being rectified, time-averaged and recorded concomitantly with the angle and force data on a Grass polygraph and a PDP-11/23 plus computer. Measurements were made prior and following either a 30 min period of PMS (exp group) or after 30 min of rest (ctl group). PMS of the plantarflexors was obtained by standing the child with the feet dorsiflexed on a tilt table for 30 min. Results indicate that PMS significantly reduced ($p<0.05$) neuromuscular responses during passive movements as demonstrated by the decrease in total work and muscle activation values in both the lengthened and shortened muscles. These effects lasted for at least 35 minutes after cessation of PMS. Both the PF torque and EMG of the agonists (TS) were significantly increased ($p<0.05$) post-PMS in the isometric tests but the DF torque and TA activation were unchanged. The decreased response in the TS during passive DF was associated with an increased torque and muscle activation ($p<0.01$) during voluntary PF. These changes were not found in the ctl group. The concomitant decrease of EMG responses during passive PF and DF suggests that the inhibitory effects of PMS are exerted through neural mechanisms; on the other hand, the effects on voluntary muscle activation are more likely to be related to more specific peripheral changes induced by the stretching procedure since they were observed in the stretched muscles only. This work was supported by a grant from NHRDP Canada.

426.7 EFFECTS OF REFERENCE AGENTS ON DECEREBRATE RIGIDITY AS MEASURED WITH A MECHANOGRAPHIC DEVICE. S. M. Yuhas*, G. W. Campbell, P. J. Barr*, P. A. Boxer and R. J. Anderson. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

The decerebrate cat has long been used as a model of rigidity, hypertonia, and spasticity. However, a consistent and reliable method for the quantitative analysis of this hyperreflexive behavior has been lacking. We have developed a mechanographic device that provides for the precise flexion of a decerebrate cat's hindlimb, with the simultaneous recording of the reflexive force produced by the limb. These experiments were designed to determine the profile of action of the antispasticity drugs, diazepam, baclofen, and chlorpromazine.

Cats were anesthetized with a mixture of halothane and nitrous oxide and tracheotomized. A bilateral carotid occlusion was performed and cannulae were inserted for monitoring blood pressure and drug delivery. Following trephination of the skull, a mid-collicular decerebration was performed. The animal was placed in a holding frame in which the femur was held rigid and permitted rotation of the lower limb about the knee joint. The ankle was attached to a rotating mechanical arm so that the knee was aligned with the center of rotation. Flexion of the limb was accomplished with a precision stepping motor controlled by a microcomputer. The net reflexive torque was calculated by averaging the digitized torques generated by 3 flexions (at a given velocity) and subtracting the residual force generated following treatment with a paralytic agent. The angle at which the reflex force was initiated the reflex threshold) was calculated by fitting a line to the linear portion of the torque-angle plots and calculating the x-intercept.

Experiments were performed at a fast (30 degs/sec) and slow (5 degs/sec) flexion speed. Measurements were made 30 min after dosing and compared to a mean of four predose readings. Control experiments in which the animals were given saline indicated that there was very little change in either the reflex threshold or maximum torque during the 4 hour duration of the experiment. Diazepam (0.1, 0.4, 2.0, 5.0 mg/kg) produced a dose dependent decrease in the reflex threshold and maximum torque at both velocities. Chlorpromazine (0.25, 0.5, 1.0, 2.0 mg/kg) also decreased these parameters in a dose dependent manner and at the highest dose almost completely eliminated the reflex-induced torque. Baclofen (0.05, 0.25, 0.5 and 1.0 mg/kg) produced similar effects in the animals but to a lesser degree. These results show that this mechanographic device can be used to reliably measure both the reflexive force and the hyperreflexia exhibited by the decerebrate cat. Furthermore, the close correlation between the results of these experiments and the clinical efficacy of these compounds indicates that this device would be an excellent tool for the analysis of novel antispasticity drugs.

426.8 THE EFFECTS OF THE NMDA ANTAGONISTS, 2-AMINO-7-PHOSPHONOHEPTANOIC ACID (AP7) AND KETAMINE, ON MONO AND POLYSYNAPTIC REFLEXES IN THE SPINALIZED CAT. G.W. Campbell, P.J. Barr*, S.M. Yuhas*, P.A. Boxer and R.J. Anderson. Warner-Lambert Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Three excitatory amino acid receptor subtypes have been identified in mammalian spinal cord. Classification is based on selectivity for the specific agonists: N-methyl-D-aspartate (NMDA), quisqualate (Quis), and kainate (KA). Pharmacological and functional characterization of Quis and KA receptors are incomplete, since no selective antagonists have been identified. However, 2-amino-7-phosphonoheptanoic acid (AP7) has been demonstrated to be a potent and selective competitive antagonist at the NMDA receptor. The dissociative anesthetic ketamine is described as a functional antagonist of this receptor subtype. These experiments were designed to examine the effects of AP7 and ketamine on spinal cord neurotransmission, to determine possible efficacy of NMDA antagonists in the treatment of neurologic disorders.

Following a C1 section, mongrel cats (2.0 to 4.0 kg) were artificially respired and halothane anesthesia was discontinued. A partial laminectomy was performed and dorsal and ventral spinal roots L6 - S1 were isolated and cut. Supramaximal stimulation (10x threshold) was applied to a dorsal root and the resulting monosynaptic and polysynaptic reflexes were recorded from a ventral root. The amplified signal was averaged and the areas under the curves integrated for analysis. Drugs were administered intravenously in saline. AP7 (3, 10 and 30 mg/kg), produced a marked and selective decrease in the polysynaptic reflex. This decrease was rapid (occurring as soon as 4 minutes after the 3 mg/kg dose) and was both time and dose dependent. Ketamine (1, 3, and 10 mg/kg) also produced a decrease in the polysynaptic reflex at each dose. The effect was short in duration, with the reflex area returning toward control levels by 20-30 minutes following dosing. Neither AP7 nor ketamine had any significant effect on the monosynaptic reflex at any dose or time.

Polysynaptic reflexes result from activation of -motoneurons via interneurons in the spinal cord. The marked decrease in the polysynaptic reflex by NMDA antagonists suggests that these interneurons release a transmitter which acts at NMDA receptors. In contrast, the lack of effect on monosynaptic reflexes indicates that NMDA receptors are probably not involved in the generation of the Ia afferent-motoneuron monosynaptic EPSP. The preferential reduction of polysynaptic reflexes suggests that selective NMDA antagonists might reduce the hyperreflexia associated with spastic disorders without exhibiting the muscle weakness liability of current anti-spasticity drugs. These results confirm earlier research indicating that both competitive and functional NMDA antagonists have a potent and selective activity in the feline spinal cord.

426.9 ALTERATION OF A WITHDRAWAL RESPONSE IN RATS: DEPENDENCE OF DEVELOPMENT AND RETENTION ON SYMPATHETIC INNERVATION AND INHIBITORY ROLE OF CENTRAL SEROTONERGIC INPUT. M.F. ANDERSON* and B.J. WINTERSON. Department of Physiology, University of New England College of Osteopathic Medicine, Biddeford, Maine 04005.

A variety of clinical conditions appear to involve hyperactive reflex loops between spinal cord segments and peripheral structures. These disorders include causalgia, Raynaud's disease, and chronic muscle spasm (somatic dysfunction). All of these disorders appear to have a sympathetic component and properties in common with animal models of reflex alteration. In the present study, the spinal fixation model, an example of reflex alteration, was used to assess the role of sympathetic activity in the generation and maintenance of a long term hindlimb asymmetry. Electrical stimulation (1.75-2 mA, 7 msec, 100 Hz for 60 min) of rat hindlimb induced a maintained flexion which was quantified by applying 0.5g weights until the stimulated and contralateral leg lengths were equal. Much of the induced flexion was lost by 72 h ("masked") and a significant amount recovered subsequent to spinal transsection at T7 ("unmasked"). Since serotonin has been implicated in the modulation of preganglionic sympathetic neurons, para-chlorophenylalanine (PCPA) was used to deplete serotonin stores.

Following stimulation, control animals exhibited 24.9g flexion. At 72 h, controls retained 6.0g flexion which increased to 12.4g after transsection. In an experimental group, administration of 0.01 mg of propranolol into the jugular sinus decreased post transsection flexion from 14.8g to 6.5g in ten min. Sympathectomy (25-30 mg/kg/day guanethidine i.p. for six weeks) resulted in a decrease in post stimulation flexion (14.0g) in comparison to controls (24.9g). At 72 h, these animals retained 7.6g and this was unaffected by transsection. Administration of PCPA (300 mg/kg i.p.) three days prior to fixation resulted in 25.9g post stimulation, similar to controls (24.9g). At 72 h, 10.9g remained and was unchanged by transsection. Administration of propranolol as described above prompted a decrease in flexion to 5.3g. A second group of PCPA treated rats was perfused with propanolol (0.01 mg/min) for the duration of stimulation. Afterward, flexion (13.0g) was markedly decreased compared to controls (24.9g). At 72 h, 8.3g of flexion remained and was unchanged by transsection.

These results suggest that: 1) Sympathetic innervation is responsible in part for development of long term spinal memory. This was demonstrated by a decrease in flexion developed in sympathectomized rats. 2) A β adrenergic mechanism is implicated, as propanolol given either during stimulation or after transsection decreased flexion. 3) Masking is due to an inhibitory descending serotonergic influence, since pretreatment with PCPA abolished masking.
(M.F. Anderson was supported by a Burroughs Wellcome Osteopathic Research Fellowship).

426.10 **STUDIES OF HINDLIMB REFLEXES IN THE NORMAL AND 6-OHDA LESIONED RAT, A NON-INVASIVE MEASURE OF THE CENTRAL EXCITATORY STATE OF THE SPINAL CORD.**
S.J. Moorman*, L.R. Whalen*, and H.O. Nornes, Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO (supported in part by NIH#534424 to H.O.N.)

The experiments were designed to investigate what effects catecholamine fibers have on spinal cord function, with specific reference to electrophysiologic parameters and correlates of hindlimb reflexes in adult rats. The goal was to design a simple, reliable, and relatively non-invasive test to measure the central excitatory state (CES) of the spinal cord in the rat. The parameters and correlates of hindlimb reflexes are useful in this regard because they are particularly dependent on CES.

Fourteen week old animals received an intracisternal injection of 250ug of 6-OHDA in 20ul of ascorbic acid saline. Lesioned animals and age matched controls were tested 2 months post lesion. Reflex testing was done 1 hr. after performing a spinal transection (at 1HP) under metophane anesthesia and in separate animals 3 days post transection (at 3DP). The reflex test consisted of a series of graded electrical stimuli applied subcutaneously to the dorsum of the foot while EMG activity was recorded from the ipsilateral biceps femoris muscle. Testing was done before and after administration of L-DOPA or Clonidine.

Without administration of drugs, reflex evoked EMG activity consisted of low threshold, short latency and higher threshold, long latency components at 3DP, in control and lesioned animals. At 1HP only the low threshold, short latency component can be elicited. Stimulus response curves (SRC) were plotted for control and lesioned animals and were similar in both groups at 3DP. In the controls at 3DP, Clonidine and L-DOPA had similar effects on the SRC of the long latency component. After administration of L-DOPA or Clonidine at 1HP in controls, the long latency component could be evoked and had similar SRC's. In lesioned animals at 1HP and 3DP, L-DOPA showed no significant effect on reflex evoked activity, while Clonidine had effects similar to those seen in the controls.

Conclusions: Reflexly evoked EMG activity provides a relatively non-invasive test of the state of the spinal interneuronal circuitry. Results from reflex tests of 6-OHDA lesioned and control animals, before and after L-DOPA or Clonidine injections confirm previously reported effects of catecholamine fibers on hindlimb reflexes (E. Jankowska et al., Acta P. Scand. 70:369-388, 1967). The reflex test reported here will prove useful in assessing functional recovery from spinal cord injury.

426.11

WITHDRAWN

426.12 THE PINEAL GLAND MODULATES BEHAVIORAL RESPONSE DIRECTION IN RATS. K.A. Dawson, D.P. Crowne* and A. Adelstein*. Psychology Department, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

The pineal gland is a median brain structure that may modulate behavioral response direction by synchronizing environmental information provided by lateralized mechanisms in the central nervous system. Spontaneous turning behavior and orientations to unilateral and bilateral visual and auditory stimulation were examined in each of 60 male Long Evans rats for one week preoperatively and for 4 weeks following one of three treatments: 1. anesthetic and scalp incision (AI control); 2. treatment 1 plus skull defect and electrode insertion(sham lesion); 3. treatment 2 plus electrolytic lesion (1 mA, 20 secs) of the pineal gland (pineal lesion). Half of the animals in each treatment group were also subjected to postoperative darkness for 48 hours and subsequently returned to the standard LD 12:12 colony room. Each animal was tested twice per day during the light phase of LD 12:12, one test between 0800 and 1300 (morning), the other between 1500 and 2000 (evening).

Performance change ratios were computed that reflected each postoperative behavioral frequency relative to the corresponding preoperative frequency. Repeated measures analysis of variance on these scores showed animals with pineal lesion to be significantly ($p<.05$) different from sham and AI controls in exhibiting a 37.5% loss of rightward orienting ability to bilateral visual stimulation and a 33% increase in rightward orienting ability to bilateral auditory stimulation. Also in comparison to both control treatments, pineal lesion significantly lowered spontaneous turns (incomplete circles) in either direction during evening test sessions. While pineal lesion also attenuated (to 17%) a morning increase (45%) in leftward turns shown by sham animals, 48 hours of postoperative darkness following pineal lesion markedly enhanced leftward turning in morning sessions (30%). The interaction between pineal lesion, test time, and postoperative environment did not appear in rightward turning behavior.

These results suggest that pineal dysfunction can produce not only visual extinction from the same side as auditory alerting but can also reduce temporally lateralized spontaneous turning behavior, the latter effect being attenuated by extended postoperative darkness.

426.13 SEROTONERGIC INFLUENCE ON THE STARTLE RESPONSE AND THE PREPULSE INHIBITION OF ACOUSTIC STARTLE. Kevin P. Nanry* and Hugh A. Tilson. (SPON: T. Sobotka). National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

The unconditioned startle response is usually elicited by tactile or acoustic stimulation, and has been used increasingly to study behavioral effects of serotonergic drugs. The present study examined the effects of several 5-HT agonists, quipizine, 5-Methoxy-N,N-dimethyltryptamine (5-DMT) and 8-OH-2-(di-n-propylamino) tetralin (8-OH-DPAT) and antagonists, ketanserin and metergoline, on the startle response and prepulse inhibition of acoustic startle. Male Fischer 344N rats were administered the putative 5-HT_{1a} receptor agonist 8-OH-DPAT subcutaneously at 0.0, 1.0, 2.0 and 4.0 mg/kg, five minutes prior to being tested. 8-OH-DPAT augmented the startle response in a dose-dependent manner. 5-DMT (0.0, 1.0, 2.0 and 4.0 mg/kg), also a 5-HT_{1a} agonist, similiarly produced an increase in startle response when given intraperitoneally five min prior to testing. Quipizine, which mainly has 5-HT_2 agonist properties, was injected subcutaneously at 0.0, 1.25, 2.5, 5.0 and 10.0 mg/kg 1 hr prior to testing. This agonist, unlike the others tested produced an overall decrease in responding. Three hours prior to testing, rats received subcutaneous administration of the two serotonin antagonists, ketanserin (0, 1.0, 2.0 and 4.0 mg/kg) and metergoline (0, 0.25, 0.5, 1.0 and 2.0 mg/kg). Ketanserin had no overall effect and metergoline augmented the response. These data indicate that the 5-HT_{1a} and 5-HT_2 agonists had an opposite effect on startle behavior and the mixed 5HT_1 and 5HT_2 properties of metergoline may be factor involved in augmenting the startle response. The prepulse inhibition data showed that none of the compounds interfered with the ability of the prepulse stimulus to inhibit responding. Further research needs to be conducted to determine the role of 5-HT_1 and 5-HT_2 receptors in the mediation of the acoustic startle reflex.

426.14 AUDITORY STARTLE HABITUATION: RESPONSE AMPLITUDE FOLLOWING ACUTE d-AMPHETAMINE, PCA OR PENTOBARBITAL CHALLENGE. J. Buelke-Sam and R. L. Bailey*. Toxicology Division, Lilly Research Labs, Greenfield, IN 46140.

Drugs that increase dopamine (DA) transmission have been shown to increase startle responding. Davis (1984) has reviewed the data concerning d-amphetamine (d-A) enhancement of startle and has suggested that high doses (>3mg/kg) are required to increase acoustic startle. There is a considerable amount of data suggesting that serotonin (5-HT) exerts a tonic inhibitory effect on startle. Agents such as p-chloroamphetamine (PCA), which cause a long-term depletion of 5-HT, have been reported to increase startle at a time when such depletion is greatest (2-24 hrs after dosing). In an attempt to clarify the dose-response relationships among these agents' actions, locomotor activity in Figure-8 mazes was measured following challenge doses comparable to those used in an auditory startle habituation task. Auditory startle habituation was measured in young adult female rats and in adult male mice in SPSG chambers. The test session involved a 5-min acclimation period at background noise level (70 dB) prior to 50 presentations of 120 dB noise bursts at 8-sec intervals. One week following baseline determinations, 18 rats each were dosed IP with either 0.5, 1.0, 1.5 or 3.0 mg/kg d-A, 5 mg/kg PCA, or 15 mg/kg Na pentobarbital and retested 15 min following injection; 12 mice each were dosed IP with either 2.5 mg/kg d-A or 20 mg/kg Na pentobarbital. PCA-treated rats were tested a third time 24 hr after dosing. Locomotor activity was monitored in SPSG Figure-8 mazes for 1 hr and then 9 rats/group or 8 mice/group were dosed as above and activity monitored for an additional 2 hr. Two-hour activity was monitored in PCA-treated rats at only a single time point, 5-6 days following dosing. Doses of 0.5, 1.0, 1.5 and 3.0 mg/kg d-A increased startle amplitude in rats by 23, 28, 56 and 54%, respectively; 1.0 and 1.5 mg/kg maximally stimulated locomotor activity. A dose of 2.5 mg/kg d-A increased startle amplitude in mice by 33%. Pentobarbital did not alter startle responding but markedly reduced activity in both rats and mice. Results with PCA showed slight enhancement of startle amplitude 15 min following injection (15-25%), but no difference between baseline and 24 hr values. Locomotor activity was reduced only marginally at 5-6 days following PCA treatment. These results indicate: 1) doses of d-A below those resulting in stereotypic behavior enhanced startle in rats and mice, 2) a sedative dose of pentobarbital did not alter startle in either species, and 3) PCA-induced release and long-term depletion of 5-HT had little or not effect on startle responding in rats. These data do not support the contention that stereotypic doses of amphetamine are required to increase auditory startle amplitude, nor do they suggest that 5-HT played a large role in the modulation of startle amplitude in this paradigm.

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY V

427.1 THE SENSITIVE PERIOD FOR THE EFFECTS OF MONOCULAR DEPRIVATION IN THE ZEBRA FINCH. K. Herrmann* and H.-J. Bischof* (SPON: A.P. Arnold). University of Bielefeld, Dept. of Ethology, POB 8640, D-4800 Bielefeld 1, F.R.G.

Previous experiments with (14-C)-2-Deoxyglucose suggested the existence of a sensitive period for the effects of monocular deprivation in the nucleus rotundus of the zebra finch brain (Herrmann, K. and H.-J. Bischof, Exp. Brain Res., 64: 119, 1986). In this study we tried to determine the time course of this sensitive period by measurements of cell size and volume changes in two visual areas of the zebra finch brain, the nucleus rotundus of the thalamus and the telencephalic ectostriatum.

25 zebra finches were subjected to 40 days of unilateral eye closure starting at ages regularly spaced through the first 70 days. Immediately following the deprivation period the animals were perfused, the brains were cut and the cell size of neurons of nucleus rotundus and ectostriatum and the volume of the rotundus were estimated from nissl- stained sections. The results show, that monocular deprivation markedly affects the cell size in both areas if the treatment starts at 1 or 10 days posthatch. The differences between deprived and non-deprived cells decline monotonically with increasing visual experience prior to deprivation. However, deprivation starting at day 40 causes as severe effects as very early monocular closure. In contrast, deprivation onset at day 50 or later does not lead to abnormalities. The measurements of the volume of the nucleus rotundus parallel the results of the cell size measurements, whereby the second increase is delayed for 10 days and occurs with deprivation onset at day 50.

These data indicate, that the time course of the sensitive period for the effects of monocular deprivation may be double-peaked. The sensitivity for external stimuli declines from hatching until day 30, but has another peak at 40 - 50 day of age. The end of the sensitive period, as determined by our experiments is around day 50.

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427.2 EYE GROWTH IN CHICKENS WEARING LENSES. Frank Schaeffel* and Howard C. Howland, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

Visual deprivation due to visual field restriction in growing chickens has been shown to be very efficient in producing axial myopia (increased depth of the vitreous chamber). Apparently, this change in eye growth is not a result of a change in accommodative tonus in the deprived eye because myopia can also develop after the optic nerve has been cut (Troilo et al, Soc. Neurosci. Abstr. 12:120, 1986). However accommodation may still be important in regulating eye growth. We shifted the resting accommodative tonus in young chickens (n = 36eyes) by treating them with moderately powered, defocusing lenses for a period of 20days. Lenses were attached to small leather hoods by velcro fasteners and were thereby easily removable for cleaning every 1-2 hours. It was found by infrared photoretinoscopy (Schaeffel et al, Vision Res. 26:1977, 1986) that chickens wearing lenses could keep images focused on their retina. The treatment resulted in a consistent shift in non cycloplegic resting refractive state which was in the direction to compensate for the lens. From measurements of transscleral images in excised eyes we conclude that the posterior nodal distance (PND) was increased when negative lenses were worn as compared to eyes over which positive lenses were worn. If it is assumed that the ametropia which developed is completely axial in origin the change in the PND can be converted into an expected shift in refractive state. The calculated shift matches well the one which was, in fact, observed in the living bird. However the shift is much smaller (38-51%) than the one which could be expected if the full power of the applied lenses was compensated. From the moderate changes in eye growth and the fact that the scatter of the values of the refractive state was much smaller here than in deprivation experiments, we believe that we triggered a regulatory mechanism for eye growth with the feedback loop closed. By contrast, deprivation experiments provide open loop conditions because compensatory eye growth cannot improve the retinal image quality.

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427.3 **THE DEVELOPMENT OF OCULAR GROWTH AND REFRACTIVE STATE IN NORMAL AND MONOCULARLY DEPRIVED TREE SHREWS (TUPAIA BELANGERI).** N. A. McBRIEN* and T. T. NORTON. Dept. of Physiological Optics, University of Alabama at Birmingham, Birmingham, AL 35294.

It has been shown in several animal species that the visual environment can affect the development of ocular growth. Monocular deprivation of form vision has consistently been shown to cause a relative axial myopia (Smith et. al, 1987). This myopia has many similarities with human axial myopia and may serve as a suitable animal model. The present study investigated the development of eye growth and refractive state in lid-sutured and normal tree shrews, a species in which lid suture myopia has been shown to have many similarities with primate refractive development (McKanna et. al, 1983).

Refractive state was assessed by retinoscopy and coincidence optometry. Eye growth monitored using keratometry and A-scan ultrasonography. Tree shrews were placed in groups spaced 15 days apart and raised in a 16hr/8hr light-dark schedule. All tree shrews underwent atropine cycloplegia before refractive and structural measures were recorded under anesthesia.

Refractive development of visually normal tree shrews clearly showed the process of emmetropization found in other species, including humans. There was a rapid decrease in the marked hyperopia found at eye opening, stabilizing around +5D at 3-4 months. The growth of corneal curvature and anterior chamber depth levelled off at an earlier stage in development than other ocular parameters. Lens thickness continued to increase throughout development, while vitreous chamber depth initially increased and then underwent a reduction concordant with increases in lens thickness. The growth of overall axial length stabilized between 3 and 4 months.

The initial structural change in monocularly deprived animals was a flattening of the corneal curvature causing a transient hyperopic increase in refraction, relative to the fellow control eye. After 30 days deprivation, an increase in vitreous chamber depth was found in the deprived eye relative to the fellow control eye. This difference continued to increase until eye growth was complete, accounting for the majority of difference in axial lengths between deprived and control eyes. Anterior chamber depth was found to be slightly shallower in deprived eyes. A relative myopia first developed around 30 days deprivation and animals deprived throughout ocular development consistently showed high degrees of myopia (>10D). The results demonstrate that the tree shrew is a reliable animal model for the development of myopia and shows a similar time course of ocular growth and refractive development as primates, in relative age terms. (supported by grants EY-05922, RR-05807, EY-03039)

427.4 THE EFFECT OF ENUCLEATION ON TRANSSYNAPTIC TRANSPORT OF WGA-HRP IN A DEVELOPING RAT VISUAL PATHWAY. S.K. Itaya. Dept. of Anatomy, Univ. of Illinois at Chicago, Chicago, IL 60680.

The developing retinal projection to the ipsilateral superior colliculus (SC) in the rat provides a model system for the study of synaptic competition and cell death. Normal development can be altered by enucleation at birth, resulting in the retention of normally transient connections. Since the contralateral retino-SC pathway readily demonstrates transsynaptic transport of wheat germ agglutinin-horseradish peroxidase (WGA-HRP), transsynaptic labeling was studied in the the ipsilateral retino-SC connection after enucleation, based on the hypothesis that increased ipsilateral cell survivability and synapse maturation after enucleation would support transsynaptic labeling. Rat pups were given repeated intravitreal injections of WGA-HRP and perfused at postnatal ages of 1-45 days (P1-45; PO=day of birth). Three groups were studied: normal controls (n=16), enucleated on PO (n=29), and enucleated on P5 (n=6). Reaction product in the ipsilateral parabigeminal nucleus (PBG) was taken as evidence of transsynaptic transport at the ipsilateral retino-SC connection.

In normal cases, there was no reaction product in the ipsilateral PBG. When enucleation was carried out on PO, 1 of 11 cases was labeled in the ipsilateral PBG between P1-5, while 16 of 18 cases were labeled between P6-P45. Reaction product was typically located in the dorsal and ventral divisions of the PBG in its rostro-caudal extent. Animals enucleated on P5 showed sparse labeling in the ipsilateral PBG at rostral levels.

The results suggest that, under the experimental conditions employed, enucleation during a critical period (PO) results in transsynaptic labeling in the ipsilateral retino-SC pathway after P6, where it normally does not occur. One explanation is that the cells and their synapses which are saved by enucleation become capable of transsynaptic transport of WGA-HRP. Thus, transsynaptic labeling with WGA-HRP may provide an early distinguishing clue between surviving and degenerating cells during the poorly understood process of synaptic competition and cell death. The results fit the hypothesis that WGA-HRP labels the transport pathway of an endogenous trophic factor operating only in the neurons destined to survive. (Supported by NS21948).

427.5 ACCESSORY OPTIC SYSTEM IN THE WALLABY SETONIX BRACHYURUS: EFFECTS OF EARLY UNILATERAL ENUCLEATION. L-A. Coleman* and L.D. Beazley. Dept. of Psychology, Univ. of Western Australia, Nedlands, W.A., Australia, 6009.

Early unilateral enucleation results in an increased projection to primary visual centres ipsilateral to the remaining eye in the mature animal. Exuberant projections to the main primary visual centres, the dorsal lateral geniculate nucleus (dLGN) and superior colliculus (SC), have been well documented (Lund,R.D.& Lund,J.S., Science,171:804, 1971; Godement,P. et al.,J.Comp.Neurol.,230:552, 1984; Insausti,R. et al.,J.Comp.Neurol.,234:393, 1985). In contrast, there are few reports of exuberant ipsilateral projections to other primary visual centres, such as the accessory optic system (Sanderson,K.J. et al.,J.Comp.Neurol., 180:841, 1978; Collewijn,H.& Holstege,G.,Exp.Brain Res.,57:138, 1984). We report here changes seen in primary projections to nuclei of the accessory optic system in the wallaby, quokka, following early unilateral eye removal.

Unilateral eye removals were performed at two stages of development. The first group were enucleated on the day of birth (PO). At this stage only a few retinal axons have reached the brain; these fibres have crossed the chiasm but have yet to invade visual centres. A second series was operated at 35-40 days postnatal (P35) when visual projections were widespread and fibres from each eye overlapped in dLGN and SC (Harman,A.M.& Beazley,L.D.,Anat.Embryol., 175:181, 1986). At P100, when visual projections were mature (Harman,A.M.& Beazley, L.D., Anat. Embryol.,175:181, 1986), anterograde projections from the remaining eye were examined. Unilateral intraocular injections (15-20uL) of horseradish peroxidase (Boehringer, 25% solution with 2% DMSO), were made in six controls and six enucleated animals (three operated at PO and three at P35). After 24 hours, brains were fixed by perfusion, sectioned at 40um in the coronal plane and reacted with tetramethylbenzidine.

In controls, the dorsal (DTN), lateral (LTN) and medial (MTN) terminal nuclei received bilateral input, although in every case nuclei ipsilateral to the injected eye were weakly labelled compared to their contralateral counterparts. Tracts were clearly seen contralaterally and revealed that all three nuclei received input via the superior fasciculus (SF), while the MTN was also innervated by the inferior fasciculus (IF). In the experimental series, the most striking differences occurred in animals enucleated at PO. In PO enucleates, ipsilateral projections to all nuclei of the accessory optic system were more densely labelled than in controls and fibres of the SF, though not of the IF, were much more pronounced ipsilaterally than normal.

427.6 EFFECTS OF MONOCULAR OCCLUSION ON LATERAL GENICULATE NUCLEUS (LGN) ANATOMY AND HISTOCHEMISTRY, AND ON RETINAL DOPAMINE SYSTEM IN INFANT RHESUS MONKEYS. M. Tigges, P. M. Iuvone, J. Tigges, A. Fernandes* and J. A. Gammon*. Yerkes Regional Primate Research Center and Departments of Anatomy and Cell Biology, Pharmacology, and Ophthalmology, Emory University, Atlanta, GA 30322.

Depriving one eye of contour vision through eyelid closure during a sensitive postnatal period leads to severe anatomical and functional deficits in the geniculostriate system. The retina itself, however, seems immune to this manipulation. In an effort to determine the effects of monocular blockage of photic stimulation on LGN and retina during postnatal development, one eye of newborn rhesus monkeys was fitted with a black occluder lens. In the LGN, occlusion for 4 and 12 weeks, respectively, resulted in mild anatomical alterations. In Nissl-stained sections, the overall staining intensity appeared almost normal at low magnification. At higher magnification, however, in both hemispheres neurons in magno- and parvocellular layers connected to the occluded eye were smaller compared to their counterparts connected to the open eye. Changes were greater in the LGN ipsilateral to the occluded eye. In sections reacted for the mitochondrial enzyme cytochrome oxidase, a marker sensitive to changes in neuronal metabolic activity, parvocellular layers in the LGN ipsilateral to the occluded eye showed a slight pallor, but both magnocellular layers were of similar density. The LGN contralateral to the occluded eye appeared normal. The staining for acetylcholinesterase (AChE) was not different from normal in both hemispheres. These results indicate less detrimental effects of short-term occlusion on the LGN compared to long-term eyelid closure.

We also determined the effect of occlusion on the retinal dopamine system which has been implicated in visual information processing and in homeostatic regulation of retinal function. The concentrations of dopamine and its primary metabolite 3,4-dihydroxyphenylacetic acid were consistently lower in retinas of occluded eyes than in their paired controls. In addition, the activity of tyrosine hydroxylase, the rate-limiting enzyme of dopamine biosynthesis, was reduced in the occluded eyes.

Our data suggest that in the LGN after monocular blockage of photic stimulation from birth neuronal size is affected, neuronal metabolic activity in parvo- but not in magnocellular layers is changed, and AChE activity remained unaltered. In the retina, dopamine synthesis in monkeys may be stimulated by light, as it is in several other mammalian and non-mammalian species, and, finally, the primate retina itself reacts to deprivation by occlusion with decreases in dopamine biosynthesis and metabolism.

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427.7 PHYSIOLOGICAL AND MORPHOLOGICAL EFFECTS OF SHORT AND LONG TERM LID-SUTURE ON CELLS IN THE LATERAL GENICULATE NUCLEUS OF CATS.

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Kitten-onset visual deprivations reduce the recordability of Y-cells in the lateral geniculate nucleus (LGN) of the cat. In contrast, adult-onset visual manipulations have a selective effect on X-cells. In the present experiments we have assessed the effects of duration of infant-onset deprivation, and therefore age of subject at the time of data collection, on the physiology and morphology of cells in the LGN.

22 cats underwent lid suture at between 3-4 weeks postnatal age. 12 were studied between 5 and 16 months of age, and 10 were studied between 17 and 29 months. In 19 of these same subjects we measured soma sizes as well, to permit direct comparisons of morphological and physiological effects of lid suture. These data were compared to those from 8 normal controls. During recording sessions one eye was immobilized by transecting cranial nerves III, IV, and VI. Animals were sedated, not anesthetized during recording sessions. Cells (representing the central 5° of visual space) in the laminae innervated by the paralyzed eye were classified as X or Y based on a standard battery of tests, those in laminae innervated by the mobile eye were classified soley on the basis of latency to optic chiasm stimulation.

Cats reared with lid suture and from which data were collected before 17 months of age showed a reduction in the encounter rate for Y-cells and a reduction in average cell body size in geniculate laminae innervated by the deprived eye. In contrast, no cat from which data were collected at or after 17 months of age showed a reduction in encounter rate of Y-cells, giving the appearance of a more normal X-/Y-cell ratio. Similarly, the differences in average soma size between deprived and nondeprived laminae of the older subjects were smaller than in the younger animals. Furthermore, both the physiological and morphological differences between nondeprived and deprived laminae are correlated (r=.58, p<.02). Preliminary observations suggest that these late changes in the effect of deprivation are not a recovery of Y-cells, but may represent an additional X-cell loss typical of adult deprivations.

We conclude that the X/Y cell ratio and average soma size in lid-sutured animals is indeed related to the duration of the deprivation period, and consequently to age of the subject at the time of data collection.

427.8 FUNCTIONAL CONSEQUENCES OF INTERRUPTING PRENATAL BINOCULAR INTERACTIONS IN THE DORSAL LATERAL GENICULATE NUCLEUS OF THE CAT. C. White, L.M. Chalupa, M.A. Kirby[1], B. Lia and L. Maffei[2]*, Dept. of Psychology, Univ. of California, Davis, CA 95616, [1]Depts. Pediatrics & Anatomy, Sch. Med., Loma Linda Univ., Loma Linda, CA 92350, [2]Instituto di Neurofisiologia, Pisa, Italy.

During prenatal development of the mammalian visual system, projections from the two eyes to the dorsal lateral geniculate nucleus (dLGN) are intermingled before becoming segregated into eye-specific layers. Binocular interactions are important in this segregation process since monocular enucleation during early fetal development results in a retinal projection from the remaining eye that innervates the entire ipsilateral and contralateral dLGN. To examine the functional consequences of terminating binocular interactions, we studied the response properties of dLGN neurons in adult cats that were monocularly enucleated at known gestational ages.

Receptive field properties of single cells in the A layers of the dLGN were examined in extracellular recordings of five normal and five adult cats enucleated unilaterally on embryonic days 44, 48, 49, 55 and 58. (Gestation is about 65 days.) Cells were classified as X or Y based on tests for linearity of spatial summation, receptive field size, spatial resolution and latency to optic chiasm stimulation.

In the enucleated animals, the vast majority of receptive fields were normally shaped, exhibited an on- or off-center organization and could be classified as X or Y type. Two significant differences between enucleated and normal animals were found, however. First, on the average, X cells in enucleated animals had larger receptive fields and lower spatial resolutions than normal. (A comparison of Y cells could not be made due to small samples.) Second, in animals monocularly enucleated prior to segregation of retinal projections an abnormal ratio of X to Y cells was found. Analysis of the encounter frequency of the two cell types revealed that the change was due both to a gain of X cells and a loss of Y cells.

These functional differences could reflect alterations in the arborization patterns of retinogeniculate afferents in the prenatally enucleated animals (Sretavan et al., 1985). Such alterations might be expected if early eye removal creates a mismatch between the number of ganglion cells projecting to the dLGN and the number of target neurons in the nucleus. [Supported by EY03991 from NEI, INT-8320440 from NSF and NINCDS T32 NS07300.]

427.9 GABA NEURONS AND RECEPTORS DEVELOP NORMALLY IN DARK REARED AND MONOCULARLY DEPRIVED CATS. G.D. MOWER. Dept. of Neuroscience, The Children's Hospital, Boston, MA 02115.

Physiological evidence, based on the effects of microiontophoretic bicuculline, suggests alterations in the GABA system of dark reared (DR) and monocularly deprived (MD) cats. The present study addressed whether there are morphological changes in the visual cortical GABA system of DR and MD cats.

Immunohistochemical techniques (anti-GABA) were used to localize GABA cells. These cells were non-pyramidal in morphology and were distributed rather uniformly across all cortical layers. There were no differences in the number or laminar distribution of GABA cells between normal and MD cats. DR cats showed nearly a 50 percent increase in GABA cell density. This difference, however, could be accounted for by a comparable increase in total cellular packing density, as determined in adjacent cresyl stained sections.

In-vitro receptor autoradiography (^{3}H muscimol) was used to analyze $GABA_A$ receptors. Kinetic analyses indicated no difference between normal, DR, and MD cats in the number or affinity of receptors, when total cellular packing density was accounted. In all three preparations, receptors were most dense in layers II and III, least dense in layer V, and intermediate in layers I. IV, and VI. There was no evidence for any lateral (columnar) organization of receptors in any preparation.

These results indicate that the GABA system develops normally, at the light microscopic level, independently of the nature of early visual experience. It is possible that there are ultrastructural changes in GABA synapses that would not be detected by these techniques. Alternatively, the physiological abnormalities of the GABA system in MD and DR cats could reflect passive effects on the GABA system that are precipitated by changes in the pattern of excitatory afferent connectivity.

427.10 THE ONTOGENY OF SUBSTANCE P RETINOFUGAL INNERVATION OF THE DORSAL LATERAL GENICULATE NUCLEUS OF THE POSTNATAL RABBIT. D. Johnson and N. Brecha. Departments of Anatomy and Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

In the albino rabbit retina, about 25% of all ganglion cells contain substance P-like immunoreactivity (SP) and are likely to be involved in "ON-CENTER" pathways since their dendrites ramify in the proximal inner plexiform layer. These cells are found in all regions of the retina and project contralaterally upon the superficial part of the external layer of the alpha sector of the dorsal lateral geniculate nucleus (dLGN). In the adult, SP retinofugal axons and terminals form a narrow plexus, 120-250 um wide, which is located just beneath the optic tract. The present study examined the ontogeny of SP retinofugal innervation of the dLGN in the postnatal rabbit. Rabbit pups, between postnatal days 1 and 35, were perfused with a paraformaldehyde solution and brain sections were incubated in a rat monoclonal antibody directed to substance P. At postnatal day 1, fine caliber SP retinofugal axons and terminals are present in all regions of the external and internal layers of the alpha sector, including regions not innervated by SP retinal afferents in the adult. The SP fibers in the superficial part of the external layer are larger in diameter, show some degree of orientation and are denser in distribution than the SP fibers in the other regions of the alpha sector of the dLGN. At postnatal day 5, several separate and distinct SP fiber laminae are present due to the loss of some of the smaller diameter SP fibers from the deeper, more medial regions of the alpha sector of the dLGN. The differences in fiber morphology are more pronounced at postnatal days 5 and 10, with the fibers of the superficial part of the external layer having irregular contours and larger diameters than the remaining SP fibers. SP fiber loss continues until the adult pattern is established around postnatal day 25, with the greatest loss of SP fibers occurring after the eyes open. Following monocular enucleation at postnatal day 3, there is a complete elimination of SP fibers in the contralateral dLGN, demonstrating that these fibers are of retinal origin. The basis for the loss of SP fibers is unknown, but may be mediated by retraction of collateral branches and/or selected ganglion cell death. However, the possibility that the peptide may be transiently expressed in some ganglion cells has not been excluded. The present study demonstrates that SP retinofugal axons initially innervate the entire alpha sector of the dLGN and the majority of SP fibers are subsequently lost during development to establish the adult SP innervation pattern of the dLGN. Supported by EY04067 and a Sloan Fellowship to N.B.

427.11 MORPHOLOGICAL INDICATIONS OF METABOLIC DIFFERENCES AMONG RATS REARED IN COMPLEX, SOCIAL OR INDIVIDUAL ENVIRONMENTS: ESTIMATES OF CAPILLARIES AND MITOCHONDRIA. Anita M. Sirevaag, Devin Shafron* and William T. Greenough. Depts. Psych. & Anat. Sci. & Neural & Behav. Biol. Prog., Univ. Illinois, Urbana-Champaign, IL 61820.
Studies of occipital cortex have compared morphological measures from rats reared in complex environments (EC) with rats from social (SC) or individual environments (IC). Various studies have shown that ECs had a lower density of larger neurons with more synapses on them and that those synapses were larger in layer IV. ECs also had a greater volume fraction (Vv) of capillaries, a greater average capillary diameter and a shorter distance between capillary profiles and a random point in tissue. There was also more capillary per synapse and per neuron than in ICs. These results lead to the predictions of increased capillary branching and suggest greater mitochondrial activity in EC rats, presumably for metabolic support of larger neurons with more synapses. We have now tested those predictions. Mitochondria volume was estimated from electron micrographs and was 6% greater in EC and SC rats than in ICs ($p<.05$). The volume of mitochondria per neuron was 20% greater in ECs and 13.7% greater in SCs than in ICs. The greatest EC-IC difference in mitochondrial Vv was present in cortical layer IV (12.05 vs 11.5%) but there was no difference in Layer I (10.4 vs 10.1%). Capillary branching in the upper cortical layers was estimated by counting branch points and estimating total capillary surface area (Sv) in a 2 mm square sample that contained capillaries filled with india ink. Surface area was estimated using a new unbiased stereological method for vertical sections (Baddeely and Gundersen, 1986). In Area 17 EC rats had 24% more capillary branches per μm of cortex ($p<.05$), 16.4% greater capillary surface area (Sv) within the cortex ($p<.05$) and 37% more branches per m of capillary than IC rats ($p<.03$). SCs were similar to ECs and differed significantly from IC rats on all capillary measures ($p>.05$). These results show that the metabolic demand of larger neurons with more synapses can be met by the mitochondria and capillary network. The greater number of branches per surface of capillary suggests that new capillaries are budding off to increase the surface area of the network.

	EC	SC	IC	EC vs IC
Mitochondria Vv	11.6%	11.9%	10.9	$p<.05$
Mitochondria/neuron	1599 μm^3	1484 μm^3	1280 μm^3	$p<.05$
Cap surface area/μm^3 (Sv)	73.9 μm^2	68.8 μm^2	61.8 μm^2	$p<.05$
Cap branches/μm^3	45.4	43.6	34.4	$p<.05$
Cap branches/Sv	11.2	10.1	7.1	$p<.03$

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427.12 SAMPLE SIZE AND STATISTICAL POWER IN SOME MORPHOMETRIC PROBLEMS RELATED TO BRAIN DEVELOPMENT AND AGING. N Jeanpretre *, G. Leuba *, R. Kraftsik * and J.-M. Fritschy * (SPON : J.-P. Hornung) Institute of Anatomy, University of Lausanne, 1005 Lausanne, Switzerland.

In development and aging, changes in morphological parameters may go undetected due to inadequate sampling. The concept of power of a statistical test, associated with the type II error, may be used to avoid such erroneous conclusions. We have devised a program that allows computation of statistical power and appropriate sample size in the context of hierarchical analysis of variance. The problem is that of stagewise sampling when the number of individuals as well as the number of observations per individual have to be determined. Several examples are given, based on existing data, firstly, to demonstrate the calculation of statistical power for morphological changes considered to be of interest in development and aging, and, secondly, to illustrate a satisfactory strategy for the choice of sample size, both in terms of statistical validity and of economy. In an analysis of neuronal densities within a perpendicular column of cortical tissue in human area 17 (Leuba,G. and Garey,L.J., Hum. Neurobiol. 6:11, 1987), it turned out to be more important to increase the number of individuals than that of observations per individual, in order to obtain greater precision and power. In an analysis of spine densities on given dendritic segments, in visual cortex of both mouse (Leuba, G., Neuropathol. appl. Neurobiol. 9:467, 1983) and human (Michel, A.E. and Garey, L.J., Hum. Neurobiol. 3:223, 1984), this holds as well, but to a lesser extent. However in a study of the lengths of terminal dendritic segments in the lateral geniculate nucleus of the marmoset (Fritschy, J.-M. and Garey, L.J., submitted), it seemed more important to increase the number of observations per individual than that of individuals.

427.13 TRANSIENT ACETYLCHOLINESTERASE ACTIVITY IN THE VISUAL CORTEX OF THE NEONATAL CAT. A. J. Elberger and M. C. Dixon*. Dept. of Anatomy and Neurobiology, College of Medicine, Univ. of Tennessee Memphis, Memphis, TN 38163.

Transient acetylcholinesterase (AChE) activity has been reported in rodent, cat and primate, in visually and non-visually related areas, in cortical and subcortical regions. The present study investigated whether visual cortical areas 17, 18 and 19 in the cat showed developmentally related transient AChE patterns.

Cats at postnatal day (PND) 4, 7, 10, 12, 14, 16, 18, 23, 27, 30, 32, 38 or 45 (day of birth = PND 0) were perfused with 10% formalin and the brain was frozen sectioned coronally at 60 um. The following treatments were used: A)Nissl stain, B)incubation in a mixture with acetylthiocholine iodide to show all cholinesterase activity, C)incubation in B plus iso-OMPA to inhibit nonspecific cholinesterase activity, D)incubation in B plus eserine to inhibit all cholinesterase activity. In groups of four sections, one was assigned to treatment A, two were assigned to C, and one was split between B and D. Each set of sections in C were developed using one of the following: ammonium sulfite, sodium sulfide, or potassium ferricyanide. At each age tested, the developers yielded similar conclusions despite subtle differences in AChE labeling patterns. AChE activity appeared in the shape and size of neuronal perikarya indicating cell bodies, in elongated strands indicating neuronal processes, or in dissociated puncta indicating terminal fields. Corresponding Nissl stained sections were used to determine in what cortical laminae and areas the label appeared.

AChE was present subcortically at all ages in treatment B, and less in C, but never in D. At PND 4-23 no histochemical AChE activity was present in areas 17-19. At PND 27 significant AChE activity was present throughout area 17 as labeled cell bodies within a terminal field in laminae 1-2 and 5-6, and throughout area 18 with cell bodies in laminae 5-6 and terminal fields in all laminae. At PND 30 there was a tremendous intensification of activity as cell bodies and processes concentrated in laminae 2-3 and 5-6 and terminal fields in all laminae but 4, in the more central visual field representation of area 18. At PND 30 the activity in area 17 was similar to that at PND 27 with label in laminae 5-6 and more faintly in laminae 1-2; all label in area 17 was far less intense than in 18. At PND 32 labeling in areas 17 and 18 were similar to that at PND 27, and by PND 38 the AChE activity in areas 17 and 18 was not present. These results suggest either a transient population of AChE positive neurons at PND 27-32 or a transient increase in AChE activity in existing neurons during this time period. Further studies will explore possible correlations with other visual developmental events.

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427.14 **BETA ADRENERGIC RECEPTOR BINDING IN VISUAL CORTEX OF KITTENS TREATED WITH 6-HYDROXYDOPAMINE (6-OHDA): RELATION TO PLASTICITY.** **E.E. Allen, P.Q. Trombley*, and B. Gordon. Inst. Neurosci. & Dept. Psychol., Univ. of Oregon, Eugene, OR 97403.**

Intraventricular administration of 6-OHDA causes a modest decrease in visual cortical plasticity. The resulting depletion of norepinephrine (NE) cannot completely account for this change (J. Neurosci. 6:266). Kasamatsu and colleagues have reported that blocking cortical beta adrenergic receptors also decreases plasticity (Exp. Brain Res. 59:507). Because 6-OHDA treatment and blockade of beta receptors both cause supersensitivity of the beta adrenergic system in rats (J. Pharm. Exp. Ther. 203:132; J. Pharm. Exp. Ther. 207:446), we hypothesized that an increase in beta adrenergic receptor density might contribute to the decrease in plasticity. Therefore we examined the effect of 6-OHDA treatment on beta adrenergic receptors in kitten visual cortex. Subjects were given vehicle solution alone (controls; N=6), a dose of 6-OHDA which depleted cortical NE without affecting visual cortical plasticity (0.2 mg; N=7), or a dose of 6-OHDA which depleted cortical NE and decreased visual cortical plasticity (4.8 mg; N=7). Drugs were administered in either 4 or 6 single daily injections via intraventricular cannulas. Animals were sacrificed 24 hrs after the last injection. Saturation assays were performed on homogenates of visual cortical tissue using [125]I-pindolol (30-400pM). Isoproterenol (140nM) was used as a cold competitor to measure nonspecific binding. Radioactivity bound to tissue and retained on filters was measured. Scatchard plots were generated using the EBDA computer program (J. Pharm. Met. 14:213); dissociation constant (Kd) and receptor density (Bmax) were determined in multiple assays for each animal.

	controls	0.2 mg	4.8 mg
Kd(+/-SEM)	111.0 (14.5)	107.9 (8.0)	96.2 (10.9)
Bmax(+/-SEM)	49.3 (4.3)	62.5 (5.2)	54.2 (3.7)

Despite 80-95% NE depletion in both drug treated groups, neither differed significantly from the controls in either Kd or Bmax values. We conclude that 6-OHDA does not alter visual cortical plasticity by changing the affinity or density of beta adrenergic receptors. The minimal supersensitivity in kittens receiving 6-OHDA compared with that expressed in rats shows that changes in transmitter content do not always cause compensatory changes in receptor density. Perhaps 6-OHDA damaged cortical cells in a way which prevented both supersensitivity and the normal expression of plasticity.

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427.15 CHANGES IN A GAP43-LIKE PROTEIN DURING DEVELOPMENT OF THE CAT VISUAL CORTEX. H.McIntosh, K.Meiri, D.Parkinson, M. Willard & N.Daw, Depts. of Cell Biology & Physiology and Anatomy & Neurobiology, Washington University Medical School, St. Louis, MO 63110

The critical period in the development of the cat visual cortex extends from the time of eye opening to three months of age. During this time, anatomical and physiological changes may occur as a result of altered sensory input or deprivation. We decided to test whether biochemical changes might also occur in this period. Our experiments focussed on GAP-43, a growth-associated protein concentrated in growing axons that is closely related to the F1 protein. Changes in phosphorylation of the F1 protein have been associated with long-term potentiation and synaptic plasticity in the hippocampus.

Samples of primary visual cortex were collected from normal kittens throughout the critical period, aged 5 days to adulthood. Expression of membrane proteins was monitored by one dimensional (1D) and two-dimensional (2D) SDS polyacrylamide gel electrophoresis, followed by staining with Coomassie blue and/or ammoniacal silver.

Observation of 1D gels showed an age-related transition in the concentration of a few proteins. The staining intensity of one major band of about 50kD decreased with age, most notably from 40 days of age onwards. Changes were also seen in bands at 18 kD and 25 kD which increased in density from around 40 days onwards. This correlates with a possible increase in small myelin basic protein and myelin associated proteolipid protein near the time when myelin has first been detected in stains of sections of visual cortex. Membrane proteins resolved on 2D equilibrium gels showed few changes related to development. Staining of one spot decreased with increasing age. On the basis of its molecular weight and isoelectric point, this protein was identified as GAP43. When membrane proteins were separated and then Western blotted with an affinity-purified antibody raised against rat GAP43, a single band was labelled. The cat GAP43 was slightly smaller than rat GAP43 run under the same conditions. The immunostaining decreased with age, most notably beginning around 40 days, but was still detectable in adult cat visual cortex.

In summary, the concentration of very few proteins changed with age. However, we have identified a GAP43-like protein and shown that it decreases in concentration during development.

427.16 OPTIMAL TETANUS PARAMETERS FOR INDUCTION OF LTP IN RAT PRIMARY VISUAL CORTEX. R.L. Berry, T.J. Teyler, and H. Taizhen*. Department of Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Tetanus-induced long term potentiation (LTP) of evoked responses has been widely reported for the hippocampus. Hippocampal LTP is a strong candidate for a mnemonic device. Considering the likely involvement of hippocampal LTP in memory, it is surprising that there are relatively few investigations of LTP in other brain areas which might be involved in memory and other forms of neural plasticity. One such brain area that is particularly interesting is area 17 (OC1) of primary visual cortex. An enormous number of studies have focused on electrophysiological and neuroanatomical correlates of visual plasticity in area 17 of the cat. An interesting possibility is that cortical LTP might be an important mechanism for visual plasticity. Komatsu, et.al. (Neurosci. Let.,26, 1981, 269-274) reported LTP in in vitro slices of area 17 in kittens paralleling the critical period for visual deprivation effects. Our laboratory presented similar data for the developing rat at this meeting last year (Perkins and Teyler, 1986).

The present study was designed to determine the optimal stimulation parameters to induce LTP in the in vitro rat primary visual cortical slice. Female Long Evans hooded rats (2-3 weeks old) were anesthetized by hypothermia and neocortical brain slices obtained. Following 2 hours of incubation, stimulating electrodes were placed in white matter underlying area OC1M and recording electrodes in the layer II-III border to record field potentials. For purposes of comparison and to verify a stable preparation, 30 minutes of pre-tetanus baseline data were obtained. Tetanic stimulation was then delivered and the amplitude of the field potential measured every 300 sec for as long as the slices remained robust (6-9 hours). The effects of varying the frequency (2-200 Hz) and total number (120-1200) of tetanic pulses on the degree of LTP and its decay will be presented. We feel that further pursuit of this technique should give rise to valuable insights regarding the mechanisms of cortical plasticity. This research was supported in part by ONR grant #86K0664.

427.17 TOWARDS AN ORGANIZING PRINCIPLE FOR PERCEPTION: HOW HEBB-TYPE MODIFICATION INDUCES THE EMERGENCE OF FEATURE-ANALYZING FUNCTION. R. Linsker. IBM Thomas J. Watson Research Center, Yorktown Heights, NY 10598.

A Hebb-type synaptic modification rule has been shown to induce the emergence of progressively more sophisticated feature-analyzing functions, including orientation selectivity, in a layered network of cells with feedforward connections (R. Linsker, Proc. Natl. Acad. Sci. USA , 83 : 7508, 8390, 8779 (1986)). Why does this happen? Does a Hebb-type algorithm (which adjusts synaptic strengths depending upon correlations among signaling activities) cause a developing perceptual network to optimize some property that is deeply connected with the mature network's functioning as an information processing system?

I show that a Hebb-type rule causes each cell of a layered feedforward network to develop -- that is, decide with which input cells it will connect, and with what strengths -- so that the statistical variance of its output activity is maximized, subject to a constraint on overall synaptic strength. The mature cell thus performs an operation similar to 'principal component analysis,' an approach used in statistics for extracting 'interesting' structure (e.g., clustering) from complex input data.

Furthermore, given a signal-processing device, and given any particular output value from that device, one can make a best estimate (inference) of the input values that gave rise to that output. Among all devices of a certain type, I show that a suitable Hebb-type rule generates that device for which this 'best estimate' comes closest to matching the input values. I call this design optimization criterion the principle of 'constrained optimal inference' (COI). Depending on the relation between information content and signaling activity, this suggests that a device (a simplified model neuron) satisfying COI can produce output that optimally preserves input information (subject to constraints).

I demonstrate explicitly how inputs from a layer of circularly symmetric spatial opponent cells (an idealization of cell types present in cat LGN, or layer 4 of macaque striate cortex) generate orientation selectivity at the next stage of connections, as a result of this organizing principle.

It is remarkable that the principle of constrained optimal inference induces the emergence of feature-analyzing cells corresponding to observed functional types, without requiring that the network be specifically instructed -- either genetically or by rewarding 'correct' identifications -- as to what particular perceptual function (e.g., edge analysis) it should develop in a given layer. [The particular emergent function in a layer is determined by the arrangement of layers in the network and by the statistics of the environmental input (if any).] I suggest that COI is a candidate for a generic organizing principle for perceptual development. The fuller exploration of the power and limitations of this approach, and its experimentally testable consequences, is in progress.

427.18 THE HYPOTHYROID CORPUS CALLOSUM: MATURE TOPOGRAPHY IN THE PRESENCE OF EXCESS COLLATERALS. C. Gravel, R. Sasseville*, D. Blais* and R. Hawkes*[1]. [1]Dept. of Biochemistry and Lab. of Neurobiology, Laval University, Quebec, Canada G1J 1Z4.

In hypothyroid rats there is an almost complete suppression of a neurofilament-associated epitope (monoclonal antibody N210) in the axons of the corpus callosum while other cortical axons were either lightly affected or not affected at all. This deficit is reversed by T4 therapy. The absence of immunoreactivity does not reflect an absence of axons but rather their protracted immaturity: although there is a severe deficit in the number of myelinated fibers, the total number of callosal axons was found to be two times higher in P25 hypothyroid rats than in normal ones.

In area 17 of normal rats, the callosal projections are restricted to the cortex containing the binocular representation of the visual field. However, in the early stages of postnatal development the callosal afferents are distributed extensively throughout all the presumptive visual cortex with the mature pattern of alternating callosal and acallosal areas attained only at P15. It has been thought that collateral elimination during the early postnatal period is the mechanism through which callosal topography is sculpted. To explore whether the failure to eliminate excess collaterals in hypothyroid animals leads also to the retention of an immature callosal projection, callosal topography was mapped in the visual cortex of P25 rats that were made hypothyroid by propylthiouracil treatment from E17. To reveal the pattern of callosal projections, multiple injections (typically 50 nl x 10) of a 3% WGA-HRP solution were made under visual guidance into the visual cortex. Animals were perfused 48 hrs later with aldehydes and the frozen frontal sections cut at 50 um and processed for peroxidase labelling with TMB. Alternate sections were counterstained with neutral red to identify the area 17/18 architectonic boundaries. Despite extensive retention of to eliminate the excess collaterals, in hypothyroid P25 rats the callosal projections to the contralateral primary visual area as revealed by both anterograde and retrograde transport are concentrated at the borders between areas 17 and 18a, b as in the normal animals, with relatively few retrogradely labelled cells seen between these limits. Thus, although deficient in a neurofilament epitope hypothyroid callosal neurons show no obvious deficit of retrograde or anterograde transport. Furthermore the normal postnatal sharpening of the callosal connections in the visual cortex still takes place.

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427.19 **A QUANTITATIVE STUDY OF RECEPTIVE FIELD PROPERTIES IN THE CAT'S SUPRASYLVIAN VISUAL CORTEX AFTER NEONATAL LESIONS OF AREAS 17/18.** Thomas Zumbroich* and Colin Blakemore. University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

Acute ablation of areas 17 and 18 in adult cats produce changes in the properties of neurons in the suprasylvian visual cortex (Spear and Baumann, J. Neurophysiol., 1979; Guedes et al., Expl Brain Res. 1983). In newborn kittens such lesions evoke an increase in thalamic projections to the area PMLS, particularly from the lateral geniculate nucleus, but the properties of neurons in PMLS investigated so far appear to be unaffected, and this has been interpreted in terms of functional compensation during development (Tong et al., J. Neurophysiol., 1984).

We made lesions of areas 17 and 18 in 1-day-old kittens and recorded from units in PMLS after one year. For quantitative measurements, high-contrast drifting gratings or stationary, contrast-modulated gratings were presented on a display screen and spikes were collected under computer control to assess the selectivity for the direction, orientation, spatial frequency and temporal frequency of the stimulus. A separate analysis was performed for cells with peripheral receptive fields (centred at eccentricities >30 deg) and those with receptive field centres that fell within 15 deg of the area centralis and whose entire extent lay within the area of visual field retinotopically equivalent to the lesion.

The ocular dominance distribution for cells in PMLS was normal, but the responsiveness of cells with receptive fields falling in the central visual field, corresponding to the lesion, was substantially reduced. However, 68 % of cells with central receptive fields were direction selective and the distributions describing the variation in the strength of direction preference and the mean half-width of tuning were similar to those for normal adult PMLS. Tests with stationary, flashed gratings revealed that the proportion of orientation selective units amongst those cells with central receptive fields (36%) was only slightly reduced compared to unlesioned animals (45%). On the other hand, units with central fields had unusually low spatial resolution (all less than 1 c/deg) and a large proportion of them lacked low spatial-frequency attenuation ('low-pass' cells). On the other hand, some of the cells with peripheral receptive fields (outside the area corresponding to the lesion) had surprisingly high 'acuity'. Temporal selectivity appeared to be unaffected in the lesioned animals. We conclude that direction and orientation selectivity can develop normally in PMLS without the inputs from areas 17 and 18. The development of a central specialization with cells of high spatial 'acuity', however, seems to depend on functional input from areas 17 and 18.

427.20 ADULT-LIKE PROPERTIES OF INFERIOR TEMPORAL NEURONS IN MACAQUES 4-6 MONTHS OF AGE. H.R. Rodman, C.G. Gross and J.P. Skelly*. Dept. of Psychology, Princeton University, Princeton, NJ 08544.

Inferior temporal (IT) cortex is crucial for normal visual pattern perception and learning in adult macaque monkeys. However, these visual abilities, and their dependence on IT cortex, are not fully established until after the first twelve months of life. Moreover, post-natal metabolic and anatomical changes in macaque cortex persist well into the first year. These observations suggest that IT cortex undergoes significant functional maturation within the first year of life, and raise the possibility that developmental changes in the properties of IT neurons may underlie the development of visual pattern perception and learning. To address this possibility, we have begun studying IT responses in infant monkeys and comparing them to the properties of IT neurons in older subjects.

The activity of over 80 IT units was studied in three female Macaca fascicularis ranging from 115 to 195 days of age at the time of recording. Recordings were made in repeated sessions under conditions of immobilization and N_2O anesthesia identical to those used previously for adult animals. Units were tested with slits of light and dark edges and with a set of complex three-dimensional objects similar to those used for studies of adult IT. Each unit was also tested with models and photographs of monkey faces. In virtually all respects, the response properties of IT neurons in this age group closely resembled those found in the adult. The majority of IT units tested were visually driven and typically responded most strongly at the center of gaze. Most receptive fields extended into both visual half-fields, although, as in the adult, responses were usually better on the contralateral side. Selectivity for color or shape and for particular complex objects was occasionally observed. Of particular interest, cells were found that responded preferentially or only to the sight of faces. Several of these cells responded more strongly to profile views than to front views. As in the adult, responses tended to habituate upon repeated presentation of a given stimulus, but could be subsequently elicited following the interposition of novel stimuli and/or a long intertrial interval.

In summary, the response properties of IT neurons appear adult-like in the female macaque monkey by approximately four to six months of age on virtually all dimensions previously studied in the mature animal. We now plan to record IT activity in even younger animals in order to estabish the point at which properties characteristic of the adult cortex first appear.

CIRCUITRY AND PATTERN GENERATION II

428.1 RECURRENT EXCITATION OF MEDIAL GIANT FIBER COLLATERALS IN THE EARTHWORM: SUPPRESSION BY A SINGLE CONDITIONING STIMULUS. Jeffery Lee Johnson, Dept. of Physiology & Pharmacology, USD School of Medicine, Vermillion, SD 57069.

The data of Kao & Grundfest suggest that the earthworm medial giant fiber (MGF) can influence its own activity via the action of powerful recurrent excitatory mechanisms (J. Neurophysiol., 20, 553, 1957). In this study recurrent feedback circuits acting upon the earthworm MGF were investigated using the isolated ventral nerve cord. In all health preparations, recurrent excitatory postsynaptic potentials (EPSP) could be recorded following a single conducted MGF action potential. If the saline media did not contain 5 mM glucose and was not adequately oxygenated, however, the conducted MGF action potential failed to evoke any recurrent EPSPs. In several preparations (N = 25) recurrent EPSPs could last for 8-12 msec and were capable of evoking a repetitive series of low amplitude decrimentally conducted MFG collateral branch spikes. These collateral branch spikes could occur at rates in excess of 800 Hz. The recurrent excitatory effects upon the MGF collaterals invariably became blocked for a period of 14.8 ± 0.7 ($M \pm SEM$) msec following a single conducted MGF action potential. There was no evidence of an inhibitory postsynaptic potential in the MGF during the time of block, and the recurrent EPSP always became suddenly blocked at a specific P1-P2 interstimulus interval in each preparation. Thus the recurrent excitatory circuits would appear to be blocked presynaptically by separate recurrent inhibitory circuits following a single conducted MGF action potential. As a result, the recurrent EPSPs evoked by two closely spaced MGF action potentials were not capable of summating to attain threshold in order to initiate additional MGF action potentials unless the interneuronal tracts exerting excitatory effects upon the MGF were also activated. The recurrent inhibitory circuits may be of importance in preventing sustained discharge in a command neuron such as the MGF which is exposed to powerful recurrent excitatory effects. As a result, activity in the MGF could be rather precisely regulated by mechanoreceptive inputs without causing sustained after-discharge. (Drewes, Landa & McFall, J. Exp. Biol., 72, 217, 1978).

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428.2 RESPONSES OF THE ISOLATED CRAB VENTILATORY CPG TO VARIATIONS IN PO_2. J.L. Wilkens, R.E. Young* and R.A. DiCaprio, Biology, Univ. of Calgary, Canada; Physiology, Univ. West Indies, Jamaica; & Zoological and Biomed Sci, Ohio Univ, Athens, Ohio.

Ventilation of intact crabs, Carcinus maenas, in air-equil SW (PO-135 torr) is characterized by regular scaphognathite (SC) beating alternating with short and long pauses. In hyperbaric SW (>600 torr) pausing predominates, while mild hypoxia (~50 torr) stimulates hyperventilation without pauses (compensatory responses which may persist for days), and ventilation is slow and regular in deep hypoxia (<50 torr).

Ventilatory burst rates (BRsc) recorded from the SC motoneurons of the in vitro perfused thoracic ganglion are proportional to PO_2, particularly after 60 min exposures. This trend, but with greater variability, also holds for 15 and 30 min exposures.

During hyperbaric perfusion (>580 torr, 1 atmos) pausing often alternates with rapid bursting, with pausing sometimes being the dominant mode. During long pauses which may last >30 min. temporary bursting is often restored by brief trains of stimuli applied to the circumesophageal connectives. These responses are similar to those of intact crabs.

Upon switching to air-equil or hypoxic saline, BRsc's steadily decline for 30-40 min and may or may not stabilize by 60 min. At 135 torr, pauses occur infrequently and are generally of shorter duration than at 600 torr. In hypoxia, pausing is not observed and bursting is slow and regular. Even though rates are depressed, the deafferented ventilatory CPG's appear to respond to hypoxic drive, as shown above, and further exemplified by the observation that freshly dissected ganglia often remain silent for over 1 hr, but generally begin bursting soon after being switched to hypoxic perfusion.

"Compensatory" accelerated BRsc's lasting <10 min are occassionally seen following switch from air to hypoxic saline. Response latencies following switches in perfusion are 20 sec or less. BRsc's are often higher (repayment of O_2 debt?) after return to hyperbaric saline following a period at lower PO_2.

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428.3 AMINERGIC MODULATION OF GRADED CHEMICAL AND ELECTRICAL SYNAPTIC EFFICACY WITHIN THE PYLORIC MOTOR CIRCUIT OF THE LOBSTER STOMATOGASTRIC GANGLION. B.R. Johnson and R.M. Harris-Warrick. Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853.

The central pattern generator (CPG) for the pyloric motor pattern in the stomatogastric ganglion (STG) of the spiny lobster *Panulirus interruptus* consists of 14 neurons in 6 major classes whose chemical (all inhibitory) and electrical synaptic connections are known in detail. Graded chemical and electrical synaptic transmission play important roles in the coordination of the pyloric rhythm. Previous work has shown that the amines dopamine (DA), octopamine (Oct) and serotonin (5-HT) each produce a unique motor pattern from this CPG. Each amine can affect all pyloric CPG components, while each amine has different effects on different cells in the circuit (Flamm, R.E., and Harris-Warrick, R.M., *J. Neurophysiol.* 55: 847 and 866, 1986). To further understand modulatory mechanisms in this simple motor circuit we are examining amine effects on graded chemical and electrical synaptic transmission between the circuit neurons.

We measured the effects of 10^{-4}M DA, 10^{-5}M Oct or 10^{-5}M 5-HT on synaptic input-output curves for graded chemical and electrical transmission between identified pyloric CPG cells in isolated STG (19-22^{o}C). The STG were treated with 10^{-7}M TTX to eliminate spike-evoked transmission. Synaptic input-output curves were gathered by measuring the post-synaptic peak hyperpolarizations resulting from 1 sec pre-synaptic depolarizations of varying amplitudes. The following effects were seen on graded CHEMICAL TRANSMISSION: Oct had reproducibly different effects on synaptic efficacy at different synapses, including large increases, reductions, or no effect. The most dramatic increases in synaptic efficacy (up to 3-fold enhancement) were seen at PD-PY, LP-PD and VD-PY synapses. DA also had reproducibly different effects on different synapses, including reduction in PD-PY synaptic efficacy and enhancement of LP-PD synaptic efficacy. 5-HT decreased synaptic efficacy at all synapses thus far tested. The following effects were seen on ELECTRICAL TRANSMISSION: Oct had little effect on PD-PD, PD-VD, or PY-PY coupling but caused an enhancement of AB-PD coupling. DA reduced PD-PD, PD-VD and PY-PY coupling while 5-HT enhanced PD-AB coupling, reduced PD-VD coupling and had little effect on PY-PY coupling.

Although our survey of modulation by DA, Oct and 5-HT on synaptic transmission within the pyloric CPG is incomplete, we note amine effects that may contribute to the motor patterns produced by each of these amines. At least some of these effects may be targeted at specific synaptic sites rather than resulting from generalized membrane conductance changes. For example, Oct has little effect on PD-PD or PY-PY electrical coupling, suggesting little change in general input resistance, but greatly enhances PD-PY chemical transmission. A complete description of amine effects on all synapses within the pyloric CPG, combined with knowledge of amine effects on isolated cells should allow a detailed understanding of the motor patterns produced by these amines in this CPG.

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428.4 ABDOMINAL POSITIONING INTERNEURONS: ACTIVITY IN BEHAVING ANIMALS. M.L. McAnelly* and J.L. Larimer. Department of Zoology, University of Texas, Austin, TX 78712.

The stereotyped abdominal positioning behavior of the crayfish, Procambarus clarkii, has long been considered a classic command neuron system. A number of identified cells which are capable of generating full bilateral flexion or extension when depolarized have been located in the isolated crayfish abdominal nerve cord (Larimer and Jellies 1983, J.E.Z. 226:341, et. seq.). However, this work has also raised questions concerning the role of these cells as command neurons.

We have developed a tethered semi-intact crayfish preparation in order to further analyze the neural circuitry underlying abdominal positioning in an animal capable of displaying the behavior. The ventral nerve cord is dissected free from the abdomen but left attached to the cephalothorax. The cord is pinned out in a sylgard chamber which fits within a larger saline filled chamber containing the cephalothorax. A lucite block, into which a metal rod is fitted, is glued to the cephalothorax. The animal is held, via the rod with its legs resting on a movable platform. A battery driven motor lowers and raises the platform to evoke the abdominal positioning behavior. Suction electrodes on flexion and extension motor roots allow us to monitor fictive positioning behavior.

With this preparation we were able to impale interneurons to record their activity during spontaneous abdominal positioning behavior or behavior evoked by raising or lowering the platform. The vast majority of the cells that were found to evoke either extension or flexion when depolarized could not be characterized as command neurons; they were either silent during positioning behavior or, if active, hyperpolarization of the interneuron did not alter the ongoing motor output. A small number of flexion interneurons did show properties of command cells, although none was completely necessary for the positioning behavior to occur.

These results suggest that relatively few of the interneurons identified as possible command cells in isolated nerve preparations may actually have command function. Those interneurons that do function during behavior do not seem to do so independently. Since we know these interneurons interact extensively via synapses (Jellies and Larimer 1986, JCP, 156, 861) hierarchies of controlling neurons may be involved and there may be parallel groups of such neurons as well.

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428.5 SEQUENTIAL EVENTS DETERMINING THE COMPOSITE DISCHARGE OF A NEURON IN A CENTRAL PATTERN GENERATOR ARE CONTROLLED BY AN IDENTIFIED MODULATORY NEURON. P.S.Dickinson, F.Nagy and M. Moulins. Dept. of Biol., Bowdoin Coll., Brunswick, ME 04011 and Lab. de Neurobiol. Comp., Place Peyneau, 33120 Arcachon, France

Many neurons which are members of central pattern generators possess regenerative membrane properties which aid in the production of bursts of action potentials. Synaptic interactions amongst such neurons can trigger or shut off the endogenous depolarizations and allow for correct timing of their activity cycles. We have found that regenerative membrane properties and synaptic inputs interact to determine the activity pattern of the lateral gastric (LG) neuron of the gastric mill network in the stomatogastric system of the red lobster, Palinurus vulgaris. The roles of these two factors are unusual, however, in that the depolarizations due to regenerative membrane properties and due to synaptic input occur sequentially rather than at the same time, as is the case for most neurons studied to date. This results in a composite discharge in LG, comprised of two distinct phases of firing and a period of silence. The first firing phase is dependent on a regenerative depolarization, or plateau potential (it is the endogenous component of LG firing); the second is largely due to excitatory synaptic input from a rhythmic extrinsic input, the CG neuron. The relative importance of these 2 phases in determining LG's firing pattern varies considerably, from cases in which only the endogenous component produces action potentials to those in which this component is absent and action potentials occur only as a result of the synaptic activation. Functionally, this results in changes in phase relationships of discharges within the gastric network.

An identified modulatory neuron, the Anterior Pyloric Modulator (APM, Nagy and Dickinson, 1983, J. Exp. Biol. 108, 33-58), can modify LG's composite discharge by differentially altering the 2 components: APM suppresses the plateau component, but enhances the synaptic component by activating the extrinsic inputs which contribute to it. As a result, activity in APM results in a discharge uniquely due to the synaptic component. Regardless of the previous activity in the gastric network, firing in APM always leads to a gastric rhythm with defined phase relationships, namely those which characterize the 'synaptic component-only' discharge of LG. By selectively altering the sequentially occurring cellular events which underlie LG's composite discharge, an identified modulatory neuron is thus able to promote a specific motor output from a central pattern generator.

428.6 A SINGLE IDENTIFIED NEURON EXERTS SUPPRESSIVE MODULATORY CONTROL OF A LOBSTER CENTRAL PATTERN GENERATOR. J.R. Cazalets, F. Nagy and M. Moulins. Lab. de Neurobiologie et Physiologie Comparées, place Peyneau F-33120 Arcachon, France.

Production of rhythmic motor activity by central pattern generators (CPGs) is controlled by neuromodulators, as has been clearly shown in the stomatogastric nervous system of Crustacea. In this system, however, the modulators described so far act in a permissive way, i.e., they initiate or enhance the burstiness of the target network. We report here that the pyloric CPG in the stomatogastric ganglion (STG) of Homarus also receives a suppressive control which tends to diminish or to stop its rhythmic activity. This control can be exerted by a single modulatory neuron, the pyloric suppressor (PS) neuron, that we have identified. The PS cell body is located near the oesophageal ganglion, and projects to the STG. When PS is induced to fire a burst of action potentials, the ongoing pyloric activity is considerably disrupted. A 5 sec. discharge of PS, for example, is able to stop pyloric rhythmicity for more than 60 sec. Moreover, the different pyloric neurons display different sensitivities to PS effects; the LP neuron being the most sensitive, the PD neuron less so, and the neurons controlling the pyloric valve, the VD and IC, being almost insensitive. As a result, PS activity can not only stop pyloric rhythmicity for a long time, but also cause long-lasting modifications in the structure of the pyloric sequence.

PS tonic discharges induce graded alterations in the pyloric pattern that depend on the PS discharge frequency. Up to 5 Hz, PS discharge gradually changes the phase relationships and intensities of the pyloric neurons' bursts. Due to the different sensitivity of the pyloric neurons to PS effects, some of them can be selectively switched off. For instance, a 2 Hz variation in discharge of PS can induce a switch from a triphasic pattern to a biphasic pattern (by silencing the LP neuron). When PS fires at higher frequency (12 Hz), all pyloric neurons are nonrhythmic except for those controlling the pyloric valve, which display an almost unmodified rhythmic activity. Selective photoinactivation of some pyloric neurons shows that PS effects on the pyloric pattern are partly due to the long lasting suppression of the ability of the pacemaker and LP neurons to produce bursting pacemaker potentials.

In conclusion, by controlling the endogenous properties of some pyloric neurons, the modulatory PS neuron can either provoke a long-lasting suppression of the pyloric rhythm or modify the pyloric motor pattern in a graded fashion. In particular, by controlling the number of neurons that participate in the pyloric rhythm, PS can cause the pyloric neural network to assume different functional configurations. Small variations in PS firing produce shifts from one functional configuration to another.

428.7 VARIABILITY AND CHAOS: POSSIBLE NEUROINTEGRATIVE PRINCIPLES IN THE GENERATION OF RHYTHMIC MOTOR PATTERNS IN THE SEA SLUG PLEUROBRANCHAEA. G. J. Mpitsos, H. C. Creech*, C. H. Cohan, and M. Mendelson.* Oregon State University, Mark O. Hatfield Marine Science Center Newport, OR 97365.

The marine gastropod mollusc Pleurobranchaea exhibits many different oral behaviors using similar sets of muscles and nerves. One class of neurons, the buccal cerebral neurons (BCNs), lying in the buccal ganglion and projecting to the cerebropleural ganglion, has a pivotal position in the generation of all behaviors requiring coordination between the jaws/radula and the mouth and lips (Mpitsos, G. J., and Cohan, C. S., J. Neurobiol., 17: 517, 1986). The activity of the BCNs and the behaviors in which the BCNs take part in generting are quite variable (Mpitsos, G. J., and Cohan, C. S., J. Neurobiol., 17: 499, 1986). Dimensional analysis of the spike series in the BCNs, and in the motoneurons that they drive, indicates that the dynamics of the activity is chaotic. That is, while the activities of these neurons and the rhythmic motor patterns that they take part in generating are nonrandom and deterministic, they are nonetheless dissipative in the sense that activity at one time does not well predict the activity at some future time. Such variability, we believe, plays an important role in the dynamics by which different adaptive motor activity emerges from the underlying anatomic neurocircuits. AFOSR-86-0076.

428.8 MYTHS AND REALITIES OF FREQUENCY GRADIENTS AND CONSTANT PHASE LAGS DURING LAMPREY FICTIVE SWIMMING. A.H. Cohen and M.T. Baker*. Section of Neurobiology, Cornell University, Ithaca NY 14853.

Several investigators have characterized fictive swimming in the isolated spinal cord of the lamprey (e.g. refs 1,2). It has been said that one characteristic of the motor pattern is that the bursts of two ventral roots (VR) are separated by a constant phase delay of 1% of the cycle per segment as is seen in intact animals. The evidence for this is based on extrapolations from recordings of widely separated VRs. We now report that when the phase delays are computed between roots no more than 3-4 segments apart, that the delays along the cord are not constant. We can also report that when the gradient of preferred segmental oscillator frequencies is examined in detail, in the majority of spinal cords it is not (as some of us had suggested) monotonic.

The phase lags were examined in isolated spinal cord-notochord preparations of Ichthyomyzon unicuspis. Fifty segment cord pieces (of the 100 total in the intact animal) were activated by bath application of D-Glutamate (125-500uM). A method was devised whereby delays among neighboring segments could be measured; this was used along the entire length of the cord piece. The results revealed that in the middle 20 segments as well as at the rostral and caudal ends of the cord the phase delays were positive, but not uniformly 1% of the cycle per segment. Approximately 10 segments from either end the phase delays became negative (1-3% of the cycle per segment). Among the segments just rostral and caudal to the negativity, the delays rose above 1%. This pattern was seen in all the cords tested to date regardless of the pattern of segmental frequencies they exhibited.

Preliminary data had pointed to frequency differences along the chain of segmental oscillators of the locomotor CPG. It was proposed by both Cohen (ref. 3) and Grillner (ref. 2) that this was indicative of a monotonic frequency gradient along the cord. This was examined in the same cords used for the phase studies. The cords were partitioned into 4 regions with vaseline seals. Each region was activated with D-glutamate independently while the others were kept quiescent. The cord was cut at the seals and the frequencies retested. In the majority of cords there was no monotonic gradient of frequencies. One had an ascending gradient (caudal fastest) and one had a descending gradient (rostral fastest), but the bulk had some more complex pattern of differences. Typically, along the cord the frequencies first rose and then fell. They did not appear to be random or erratic, and they were consistent over time.

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Refs: 1. Cohen and Wallén, Exp. Brain Res. 41:11(1980); 2. Grillner et al., TINS, 10:34(1987); 3. Cohen, J. comp. Physiol. 60:181(1987).

428.9 COMPARISON OF FORWARD AND BACKWARD WALKING IN CHICKS. A. Bekoff, P. Feucht*, R.M. Johnston* and H.E. Settles*. EPO Biology Dept., University of Colorado, Boulder, CO 80309.

It has been suggested that a switch from forward to backward walking involves a major change in the coupling of hip with respect to knee and ankle (Miller, *et al., J. Physiol.*, 277:45P, 1978; Grillner, *Science,* 288:143,1985; Smith, *et al.,* In: *Development and Plasticity of the Mammalian Spinal Cord,* 1986). Specifically, it has been proposed that the hip extensors, which are coactive with knee and ankle extensors during forward walking in the cat, may be coactivated with knee and ankle flexors during backward walking. However, a recent kinematic analysis of human walking has shown that backward walking is not achieved by a simple 0.5 phase shift in hip-knee coordination (Vilensky, *et al., Soc. Neurosci. Abstr.,* 12:878, 1986).

We have examined forward and backward walking in chicks to determine whether hip muscle activity patterns show a phase shift of 0.5. EMG electrodes were implanted in 6 leg muscles of 0- to 4-day old chicks: gastrocnemius lateralis (GL, an ankle extensor), tibialis anterior (TA, an ankle flexor), femorotibialis (FT, a knee extensor), iliofibularis (IF, a knee extensor), caudilioflexorius (CF, a hip extensor and knee flexor) and sartorius (SA, a hip flexor and weak knee extensor). Recordings were made during forward and backward walking on a treadmill as well as during free walking.

Numerous differences are seen in cycle period, burst duration and phase of activation of the muscles. However, although phase shifts in hip muscle activity are seen, they are far less than 0.5. That is, the hip extensor remains coactive with knee and ankle extensor bursts in backward as well as forward walking. Kinematic analyses, which are currently underway, will allow us to relate the EMG patterns to the actual movements performed during forward and backward walking. Supported by NIH grant NS 20310.

428.10 EFFECTS OF DISINHIBITORY AGENTS ON SPONTANEOUS BURSTING PATTERNS OF MOUSE SPINAL CORD MINI-CULTURES. M.H. Hightower* and G.W. Gross, Department of Biological Sciences, Center for Network Neuroscience, P.O. Box 5218, North Texas State University, Denton, TX 76203.

Monolayer cultures from dissociated spinal tissue were grown on 1.5mm diameter adhesion islands centered on a fixed recording matrix of 36 photoetched indium tin oxide electrodes. These networks, ranging in size from 200 to 600 neurons, produce vigorous spontaneous activity that is usually coordinated between different electrodes and is often organized in the form of patterned bursting. As a first step in network characterization, we have investigated the contributions made by the inhibitors glycine and GABA. These influences were assessed by bath addition of the transmitters themselves and their antagonists strychnine, bicuculline, picrotoxin, and penicillin.

Multielectrode activity generally ceased following applications of 50μM glycine or 50-100μM GABA. This demonstrates a capacity of either inhibitory transmitter to halt spontaneous bursting as well as random single-spike firing. Disinhibition reversed these effects and produced more complex results that revealed similarities with the electrophysiological descriptions of epileptic seizures. Application of 1-10μM strychnine, 20-50μM bicuculline, 50-100μM picrotoxin, or 100-1000 units of penicillin usually resulted in a brief period of paroxysmal activity (<1 min, corresponding to seizure onset). A few high-amplitude bursts then preceded a period of tonic activity. This "tonic seizure" was followed by bursting activity ("clonic bursting") which frequently became rhythmic. The order of effectiveness for producing rhythmic bursting was bicuculline > strychnine > picrotoxin > penicillin. Cultures which exhibited random bursting, with wide variations in firing intensity and duration prior to drug addition, developed much more regular activity after disinhibition. Burst frequencies in slowly bursting cultures (<20 bursts/min) tended to increase after disinhibition, while those of rapidly bursting cultures (>40 bursts/min) decreased. Dual exposure to strychnine and bicuculline resulted in augmentation of the above responses which could not be duplicated by increasing the dosage of a single drug.

Although the antagonists act by different mechanisms at different receptors, their effects on network bursting are similar. However, the degree of response of specific cultures to GABAergic and glycinergic antagonism was variable, indicating differences in the neuronal culture populations. Since substantial disinhibition does not result in prolonged paroxysmal activity but produces regular rhythmic bursting, we conclude that rhythmicity is an intrinsic characteristic of small networks with predominantly excitatory interconnections.

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428.11 COMPARISON OF STEP CYCLE TIMING IN THE ISOLATED MOUSE SPINAL CORD-HINDLIMB PREPARATION WITH THAT IN INTACT, FREELY MOVING MICE. M.H. Droge, H.L. Stewart* and P. Hernandez*. Dept. of Biology, Texas Woman's University, Denton, TX. 76204.

From previous ventral root recordings, we know that combined spinal cord-hindlimb explants from neonatal mice (birth to 5 days old) are capable of generating motor rhythms (Droge et al., Brain Res., submitted) and that their phasic activity is similar to that recorded from dissociated cell cultures of fetal mouse spinal tissue (Droge et at., J. Neurosci. 6(6): 1583, 1986). The objective of the present report is to relate the timing of a normal step cycle in freely moving neonatal and adult mice to the rhythmic motor activity observed in the explant preparation.

Electromyographic (EMG) recordings were made primarily from the gastrocnemius and tibialis anterior muscles of neonatal and adult Balb/C mice. The intact neonates were from the same age group as those used for the explant studies (birth to 5 days old). In some cases, data for the intact and explant preparations were obtained from the same animal. Electrode placements remained the same. The normal step cycle in intact neonates ranged from 300-1100 ms, which was comparable to motor rhythms recorded from the explanted system. Spontaneous stepping, however, occurred far less often in the isolated spinal cord-hindlimb preparation and the cycle periods tended to be at the slower end of the range found for intact neonates. Compared with neonates of either category, freely moving adult mice tended to have faster step cycles with a range of 150-650 ms.

These data demonstrate that an isolated spinal cord-hindlimb of a neonatal mouse can generate normal step cycles for that age group. Although these cycles tend to be slower than those of freely moving adult mice, there is considerable overlap. Such evidence provides an important link between the behavior of the intact animal and future data obtained from the explant preparation. In addition to characterizing further the timing of the step cycles, we are also pursuing a systematic reduction of the isolated mouse spinal cord to determine the minimal lumbosacral explant capable of pattern generation.

428.12 Voltage clamp frequency domain analysis of NMDA activated spinal neurons. L. E. Moore, R. H. Hill & S. Grillner, Nobel Institute for Neurophysiology, Karolinska Institutet, S-104 01 Stockholm, Sweden and Department of Physiology & Biophysics, University of Texas Medical Branch, Galveston, Texas 77550, U.S.A.

Most studies of the quantitative effects of excitatory amino acids on receptor subtypes has generally been restricted to isolated or cultured neurons. These receptors are likely to be involved in the mechanisms of rhythm generation and thus need to be observed in a partially intact system showing physiological behavior. The receptor subtype responding to N-methyl-D-aspartate (NMDA) of the lamprey spinal cord fullfills this condition by eliciting fictive locomotion if suitably activated (Brodin, Grillner, & Rovainen, 1986; Dale & Grillner, 1986). NMDA induced voltage oscillations oscillations in the presence of TTX (Grillner & Wallen, 1985) are essentially abolished by the potential control of the voltage clamp. Therefore, a number of the receptor sites responsible for the voltage oscillations are electrotonically close to the soma. In the presence of NMDA and TEA net steady state inward currents can be observed at moderate depolarizations. Under these conditions, current voltage plots reveal a negative slope conductance in the potential range of the inherent oscillations. A kinetic analysis of the voltage dependent conductances is possible if the electrotonic structure of the cell is taken into account. Such an analysis was done with a frequency domain technique (Moore & Christensen, 1985) using a white noise stimulus to linearly perturb the membrane potential over a wide range of frequencies. The analysis revealed that the induced negative conductance leads to a response which is nearly 180 degrees out of phase with the stimulus. This is an unstable condition which leads to the depolarizing phase of the induced oscillations. The repolarizing phase is the result of an activation of a positive conductance which manifests itself as a resonance suggesting that an oscillating neuron can inherently entrain certain frequencies. Measured impedance functions showed resonance behavior and exhibited phase behavior characteristic of a negative conductance. Synaptic integrative responses were evaluated by computing impulse responses from the measured transfer functions.

Brodin, L., Grillner, S.& Rovainen, C.M. (1985) Brain Res.325:302-306.
Dale, N. & Grillner, S. (1986) J. Neurosci. 6:2653-2661.
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Moore,L.E. & Christensen,B.(1985)J.Neurophysiol.53:636-651.

428.13 BURST PATTERN ANALYSIS: AN INTERACTIVE COMPUTER PROGRAM FOR SIMULTANEOUS, REALTIME PROCESSING OF MULTICHANNEL ELECTROPHYSIOLOGICAL DATA. D. Raymond*,M.H.Hightower*,S.P.Fracek,Jr., R.Schafer and G.W.Gross, Center for Network Neuroscience, North Texas State University, Box 5218, Denton, TX 76203.

The analysis of multichannel electrophysiological data presents formidable throughput and storage problems. Of the several strategies for data reduction that can be employed, we have found one interim solution to be unusually effective and economical. This approach ignores individual action potentials and concentrates on burst parameters such as burst frequency, period, and duration. Furthermore, the composition of the firing pattern within bursts can be partially preserved by integrating the activity and retaining the envelope.

Our current system accepts up to 32 input channels. The program contains seven components: data acquisition; filtration; compression; integration; burst identification; statistics; and display. The system accomplishes this task through a multiprocessor network containing 3 special purpose and 2 general purpose processors. Data acquisition is accomplished by a processor with a 12 bit analog-to-digital converter. The information is synchronized through a sample and hold system and is then placed into a racetrack buffer in memory. The data is digitally filtered by an adaptive AC filter which learns the nature of the AC through continuous reinforcement. Thus, the filter adapts to changes in the AC noise level, but will not remove 60-cycle components of a random event, such as an action potential.

The filtered data is compressed by a factor of 64:1 via a sum of squares algorithm. The resulting value may be considered to represent the square of the energy present in the time interval. The waveforms of individual spikes are lost. Display routines allow for the viewing of several data channels at any point after compression. An interactive display is critical for adjustment of system parameters, including integration level, burst identification variables, and statistical reporting intervals.

The compressed data is integrated via a digital representation of an R-C circuit. The integrated data is used for analysis and description of bursts variables, including duration, amplitude, and interval. Analysis parameters are adjustable for optimization of the reporting routine. Burst information is then used to calculate the mean and standard deviations at regular intervals. Additional statistics are being developed for determining cross correlation between channels. The versatility of this software package allows its adaptation to a variety of neurobiological problems requiring massive data acquisition, manipulation, and statistical testing.

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428.14 SYNETSIM 3.0: A MODEL FOR SIMULATING RESTRICTED NEURAL NETWORKS D.K. Hartline, Bekesy Lab., Univ. of Hawaii, Honolulu HI 96822

The SYNETSIM series of neural network models has been developed for the realistic interactive simulation of small networks of individually distinct neurons using parameters measured physiologically. Earlier models of the series were limited by simplifications made to facilitate derivation of model parameters and by computer memory and speed. SYNETSIM 3.0 is more advanced and versatile, written in BASIC to run on various microcomputers, and capable of simulating a variety of electrical and biochemical integrative properties of neural networks.

SYNETSIM 3.0 includes the following design features:

1. **Compartmental structure**: The network consists of an array of compartments treated uniformly in model computations. Each is characterized by a capacitance, a leak resistance, and a leak battery. In addition, any number of active "branches" may be included to generate appropriate electrical properties. **Cells** are constructed through resistive coupling of appropriate compartments. **Electrotonic connections** between cells are generated similarly. A parallel set of "diffusionally" connected compartments is used for chemical components.

2. **Current-based representations** are used for synaptic interactions, for active responses, and potentially for spike-generating regions (previous SYNETSIM versions have been voltage based). This improves the accuracy of the simulations, although deriving parameters is more difficult than for a voltage-based representation.

3. **Synaptic interactions**, both spike-mediated and non-spiking chemical ("chemotonic") operate through a simulation of **transmitter released**. Facilitation/antifacilitation of spike-mediated PSPs and inactivation of Ca^{++}-dependent chemotonic transmitter release are featured.

4. **Active responses**, such as plateau potentials and delaying mechanisms are simulated (as in earlier versions) by Hodgkin-Huxley type representations. Modular branch paradigm coding permits easy addition of user-defined algorithms.

5. **Modulation** of the parameters of any compartment can be effected through second messengers. This provides extreme flexibility in how integrative capabilities can be simulated.

6. **Second messengers** of three kinds are simulated: a) those produced by voltage-gated processes (e.g. Ca^{++}); b) those dependent on transmitter (or hormone) binding; and c) those dependent on other second messengers (e.g. internally-released Ca^{++}; enzymatically activated pathways). This formulation can also be used to simulate activation of electrogenic pumps. Reaction chains of quite general character are accommodated.

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428.15 THREE-DIMENSIONAL GRAPHICS FACILITATES USE OF INTERACTIVE NEURAL NETWORK COMPUTER SIMULATION.

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Advances in the theory of neural networks have implications for understanding the functioning of the nervous system. Our experiences in neural network simulations reveal the need for a flexible processing environment for modeling and a graphics display to study the properties of these models. Ongoing studies in this laboratory have explored the application of three-dimensional graphics techniques to anatomical analysis of serial sections through the development of the program **CARP** (Computer Aided Reconstruction Package). This package provides graphics tools for the display of multidimensional phenomena, as occur in neural networks.

We have designed a neural network simulation package called XNET which supports models based on the principles found in contemporary research on parallel distributed processing. The XNET design provides for the generation and editing of a variety of models. Generation of a particular model requires the user to define a set of connected conceptual units, each unit of which is made up of inputs, connection weights, an activation value, and an output. If units are thought to represent neurons, inputs might be defined as the incoming firing rate to the neuron, weights defined as the effectiveness of a synapse on the neuron, activation values defined as the sum of the effects of the weighted inputs on the neuron, and the output defined as the firing rate of the neuron. The equations for the simulation of the model rule how these parameters interact, and the editing facility allows the user to change selected parameters at any time.

Three-dimensional graphics representations of the dynamic characteristics of units in a simulation use continuously-updated visual cues to inform the user about the current magnitudes of the inputs, weights, activation value, and output of each of those units. Shape of symbols, screen position, color and intensity of characteristics of units are used as visual cues for properties of a unit. The wiring diagram of input fibers, classes of responding neurons, interneurons, interconnections and output fibers can be represented in a window with color-coded lines and symbols in three dimensions. Hidden surface techniques help to cue for position of various parameters organized in three dimensions. This graphics display is generated on a Data General MV/8000 II computer with an Adage 3000 color graphics system.

Long term goals of this modeling and visualization effort are manifold. A task will be to develop models of neural circuits in the rat basal ganglia and cerebellum. We expect to incorporate more neuron-specific properties of units (action potentials, membrane time and space constants, ionic current flow, etc.) in the simulation package. We will also assess the use of multiple processor hardware as a host for the simulation package.

Support from the Biological Humanics Foundation.

428.16 DARWIN MACHINES: SELECTION AMONG STOCHASTIC SEQUENCES IN PARALLEL COMMAND BUFFERS AS A MIMIC OF CONSCIOUSNESS.

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Some aspects of "consciousness" are readily subsumed under categories widely shared in the animal kingdom: awareness under perception or sleep-wakefulness, simple behavioral choices under motor programs and hypothalamic-level motivational mechanisms. But humans spin scenarios which explain the past and forecast the future, exhibiting insightful behaviors that suggest much internal simulation of situations before overt actions begin, in addition to the more commonplace feedback-guided actions towards a goal.

Darwin Machines are my name for a class of computational automata (both biological and artificial) in which standard Darwinian random variation and environmental selection is practiced off-line, randomly generated sequences (not genes in this case but strings of modular movements, words, memories) being subjected to selection on the basis of innocuous memorized environments rather than real-time noxious ones, in milliseconds rather than millennia. Imagine a railroad marshalling yard, trains being randomly assembled on many tracks but each judged for reasonableness on the basis of memory, the sequence with the "highest grade" being selected to be let loose on the main track as one's next act. These candidate sequences might be modular motor commands for a ballistic movement such as throwing or hammering (which require serial command buffers because feedback is too slow to be effective); strings of words for a possible sentence; memory schemata for constructing a scenario that connects past and future in some sequence (if well-ordered, we may call this logic; if grading against memory is slipshod, the resulting sequences may be no more orderly than one's dreams).

The random origins could be readily obscured by wide choice (many tracks) and many repeated cycles which shape up survivors before overt action is taken. Just as many body structures (optics of the eye, Fourier transforms in the inner ear, etc.) initially seem too logical to have arisen by chance, so our best thoughts may bear few remaining signs of their stochastic sequencer origins (except, interestingly, for our notion of "free will" -- the perception that we actively make choices -- which is a natural outcome of this model). While dominant hemisphere premotor cortex has the motor sequencing reputation which suggests a "final common path" for this sort of stochastic sequencing, the candidate sequences might well be associated with prefrontal and temporal lobes, or even with basal ganglia and cerebellum.

Just as in biological evolution, evolutionary rates are likely to be a key consideration in Darwin machines: the analogs of isolation (how many separate tracks?) and speciation (how is backsliding prevented?), but also the duration of the simulation (premature closure vs. never making up one's mind).

MOTIVATION AND EMOTION II

429.1 2-DEOXYGLUCOSE STUDIES OF COCAINE AND SELF STIMULATION IN RATS.

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Kornetsky and Esposito (Fed. Proc., 38:2473, 1979) reported that cocaine decreased the threshold for reinforcing intracranial self stimulation in rats. They suggested that this drug effect represents a sensitization of the neural substrate involved in reward behavior. If such sensitization is represented by changes in the energy requirements of neurons, the location and intensity of this drug/self stimulation interaction might be visualized using the 14C-deoxyglucose (2DG) method (Sokoloff, et al, J. Neurochem., 28:897, 1977). We have initiated a study to test this hypothesis. Our first group of rats is implanted, unilaterally, with bipolar stimulating electrodes in the ventral tegmental area. At 1 week following surgery, the rats are trained to press a lever to receive a 250 m sec train of sine-waves. When the rats reach 70-80 presses/min, they are given saline (ip), then 2DG, and allowed to press for 45 minutes. Stimulation current is at threshold levels, and the number of presses/min are recorded. Group 2 is treated identically to 1 except that cocaine (10 mg/kg, ip) is given at 10 min prior to 2DG. Stimulation thresholds and presses/min are matched between the rats receiving saline and cocaine. Group 3 is trained to self stimulate, then is extinguished from this behavior. 2DG is administered in the absence of stimulation. Group 4 receives cocaine (10 mg/kg, ip) without any stimulation. Group 5 receives saline without any stimulation. Preliminary results indicate that cocaine alone, (similar to London et al, Neurosci. Lett., 68:73, 1986), decreases metabolism in the lateral habenula, and it may slightly increase globus pallidus and substantia nigra metabolism. Self stimulation/saline, similar to Esposito et al (Proc. Nat'l. Acad. Sci., 81:635, 1984) appears to increase metabolism in the ventral tegmental area, substantia nigra, dorsal raphe, and globus pallidus. Lateral habenula metabolism is not different from unstimulated controls. In rats given cocaine and stimulation, metabolism may be decreased in the lateral habenula and in the dorsal raphe as compared to saline/stimulation rats. These metabolic rates are closer to those in cocaine, unstimulated rats. Metabolism in the globus pallidus may be increased in cocaine/stimulation rats, beyond the increases seen in saline/stimulation or cocaine only rats. Additional data will confirm or refute these preliminary results. Supported by grants #NS00979 and #MH37916.

429.2 OPIATE WITHDRAWAL MOTIVATION REFLECTED IN DEFENSIVE BURYING: VALIDATION OF THE PREFERENCE CONDITIONING MODEL

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Rodents avoid places and tastes paired on only one previous occasion with precipitated opiate withdrawal, suggesting the use of preference conditioning procedures as an efficient model of motivation produced by opiate withdrawal (Mucha etal, NIDA Monogr. 75:567, 1986). The present study was necessary to test whether the simple responses in this paradigm indeed reflect the aversive property of withdrawal, since 1) opiate withdrawal signs are known from Wikler's work to undergo conditioning and 2) the behavioral demands of taste and place aversions are simple (ie., inhibition of approach responses) and, therefore, may be confounded by conditioned somatic signs of withdrawal. Accordingly, we tested this hypothesis with the defensive burying model, as there is an approach component in the rat's response to a conditioned aversive stimulus (Wilkie etal JEAB 31:299, 1979).

The protocol for conditioning of opiate withdrawal was almost identical to that previously used for place conditioning (Mucha etal 1986), except that the conditioned stimuli were not two distinct environments but two objects (a 2.5 cm sphere and a similarly-sized cube). Rats implanted with a morphine pellet 4 days earlier were injected on 1 to 3 occasions with naloxone (0.1 mg/kg, SC) and placed for 30 min in a clear Plexiglas box with either the cube or the sphere on its grid floor and on a similar number of occasions with saline and placed in the box with the alternate object.

Rats were tested for 15 min in box (43x43x60 cm) with a 4 cm layer of sawdust on the floor and the two test objects placed at opposite corners. As seen by Pinel and co-workers for shock and LiCl, they showed a clear defensive response to the object paired with the withdrawal by selectively burying it. Measures of the sawdust around the objects and the time spent burying confirmed this. Since the burying response underwent extinction, was proportional to the number of training trials, was retained for over 40 days after training, and was not seen in placebo-pelleted or naive rats, we concluded that it reflected conditioned aversive effects of the withdrawal precipitated by naloxone.

Therefore, preference conditioning produced by opiate withdrawal likely reflects the motivational property of the withdrawal.(Support: MA-9552-MRC and A-2000-NSERC).

429.3 EVIDENCE FOR A DIRECT AXONAL LINK BETWEEN REWARD-RELEVANT NEURONS IN THE VENTRAL TEGMENTAL AREA-POSTERIOR HYPOTHALAMUS AND THE MEDIAL MESENCEPHALON. S. Boye and P-P. Rompré (SPON: R.A. Wise). Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec H3G 1M8.

A recent mapping study has shown the existence of a high concentration of reward-relevant neurons in the medial mesencephalon (Rompré and Miliaressis, Brain Research, 359, 246-259, 1985). The results of this mapping study raise the possibility that reward-relevant neurons directly link the rostral and caudal mesencephalon. One way of testing this hypothesis is to use the behavioral adaptation of the collision technique developed by Shizgal and colleagues (J. comp. physiol. psychol., 94, 227-237, 1980) to determine whether the axons of reward-relevant neurons directly link two distinct brain sites that support self-stimulation. They used the collision method to demonstrate such a link between the lateral hypothalamus and the ventral tegmental area. The aim of the present study was to extend this finding by investigating the existence of a direct axonal link between reward-relevant neurons in the ventral tegmental area and the caudal mesencephalon. Male hooded rats were implanted with a fixed monopolar electrode in the ventral tegmental area, and a moveable electrode in the caudal mesencephalon. The collision test was performed once self-stimulation behavior was stable on both electrodes. The collision test consisted of the delivery of trains of pairs of stimulating pulses, a conditioning or C pulse and a test or T pulse. The C pulse was delivered to one electrode, and the T pulse to the other electrode. The interval between C and T pulses was varied between 0.2 and 10 msec. Collision was inferred from a significant increase in self-stimulation threshold at short C-T intervals. If collision was not found at the first tested site on the moveable electrode, the electrode was lowered by 0.16 mm and a new collision test was performed. Evidence of collision was observed in six animals: the self-stimulation threshold increased at C-T intervals shorter than 1.2 msec. Histological analysis performed on five brains revealed that all but one rostral electrodes were located in the ventral tegmental area and the caudal electrodes were located in the medial mesencephalon, in or near the central gray. The remaining rostral electrode was found in the posterior hypothalamus. These results provide evidence for a direct axonal link between reward-relevant neurons in the caudal mesencephalon and the ventral tegmental area-posterior hypothalamus.

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429.4 BEHAVIORALLY DERIVED ESTIMATES OF THE CONDUCTION VELOCITY OF THE REWARD-RELEVANT NEURONS THAT DIRECTLY LINK THE VENTRAL TEGMENTAL AREA AND THE CAUDAL MESENCEPHALON. P-P. Rompré, S. Boye and P. Shizgal. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec H3G 1M8.

Using the behavioral adaptation of the collision technique developed by Shizgal and colleagues (J. Comp. physiol. psychol. 94 227-237, 1980), we have demonstrated the existence of a direct axonal link between the reward-relevant neurons in the ventral tegmental area and the caudal mesencephalon (Boye and Rompré, Soc. Neurosci. Abstr. 13, 1987). The behavioral collision test consists of delivering trains of pairs of stimulating pulses, a conditioning or "C" pulse and a test or "T" pulse. The C pulse is delivered to one electrode, and the T pulse to the other electrode in alternating fashion. The interval between C and T pulses is varied and collision is inferred from a significant increase in threshold as the C-T interval is decreased. Estimates of the conduction velocity can be obtained by dividing the interelectrode distance by the difference between the collision interval and the refractory period. In this experiment, refractory periods were estimated from five animals that showed a collision effect in Boye and Rompré's study, using an analoguous paradigm but with both pulses applied through the same electrode. Recovery from refractoriness was first evident at C-T intervals greater than 0.5-0.6 ms. Conduction velocity estimates obtained for each subject range from 4 to 10 m/s, values inconsistent with the estimates reported for the ascending noradrenergic fibers. This suggests that at least part of the substrate for brain stimulation reward that links the ventral tegmental area and the caudal mesencephalon is composed of non-adrenergic, myelinated axons.

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429.5 AUTOMATED MULTIVARIATE MEASUREMENTS OF SPONTANEOUS ACTIVITY IN RATS AND MICE: COMPARISON OF THE TIME COURSE, RELIABILITIES, AND INTERCORRELATIONS OF THE MEASURES. G. C. Teskey, K.-P. Ossenkopp, and L. K. MacRae.* Dept. of Psychology, University of Western Ontario, London, Ontario, Canada, N6A 5C2.

In the present study spontaneous motor activity in rats and mice was measured with a Digiscan Animal Activity Monitor (Omnitech Electronics, Columbus, OH). Male hooded rats (n = 24) were tested for 1 hour, 2 days per week for 3 consecutive weeks. The Digiscan System differentiated 9 aspects of the spontaneous behavior: horizontal activity (HA) - total number of photobeam interruptions, total distance travelled (TD), movement time (MT), average speed (AS), number of horizontal movements (NM), average distance of each movement (AD), vertical activity (VA) number of beam interruptions, vertical movement time (VT), and number of vertical movements (NVM). Most of the variables exhibited significant ($p < .05$) decrements in magnitude (habituation) over weeks of testing (except NM, AS, and AD). Reliabilities of the measures aggregated on a weekly basis were high ($r > .45$) except for NM and VA. Intercorrelations of the variables also tended to be large ($r > .6$) and positive except for NM, which exhibited a peculiar pattern of correlation with the other variables. Male CF-1 adult mice (n = 30) were also tested for 1 hour, 2 days per week for 3 consecutive weeks. The same variables were recorded in the Digiscan System, except that VA, VT, and NVM were not used because the mice were too small to activate the vertical sensors. The mice exhibited a different pattern of habituation in activity relative to the rats. For MT, AD, and HA habituation ($p < .05$) tended to be large in the first week with mean values of the behavioral measures showing good stability in weeks 2 and 3. The reliabilities of the variables (weekly aggregates) were greater for mice (relative to the rats) for all variables. Intercorrelations of the variables also tended to be large and positive except for NM, which again exhibited a distinctive pattern in relation to the other variables. This pattern was similar to the one found in the rat data.

The present results indicate that habituation to the apparatus is necessary to obtain stable baseline data, that rats habituate more slowly than mice, that most of the variables show good re-test reliabilities, and that NM exhibits a distinctive pattern of correlation with the other variables over test sessions.

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429.6 AGE-DEPENDENT CHANGES IN BEHAVIOR OF RATS FOLLOWING NEONATAL TREATMENT WITH CHLORIMIPRAMINE. P. R. Hartley, G. W. Vogel*, D. B. Neill*, D. Kors*, and M. Hagler*. Depts. of Psychiatry and Psychology, Emory University, Atlanta, GA 30322.

Neonatal treatment with the antidepressant chlorimipramine (Anfranil) has been reported to modify the behavior of rats when adults (Mirmiran, Brain Res., 1981, 204: 129-146). However, File and Tucker (Neurobeh. Tox. Terat., 1983, 5: 3-8) were unable to replicate these results. We report here that the magnitude of the effect may be related to the age of the animal when tested.

Male Sprague-Dawley (Harlan/Sprague-Dawley) pups born in our laboratory were cross-fostered into litters of 6-8. One half were subcutaneously injected twice daily with 15 mg/kg chlorimipramine from postnatal days 8 to 22. Control animals were injected with the saline vehicle. After weaning at age 28 days, they lived in groups of 5. A reversed lighting cycle was used; all behavioral testing was conducted in the second half of the dark phase.

Open field activity was examined in a circular arena, 82 cm diameter, white in color with a 25 W bulb hanging 61 cm above the floor. These tests were 2 min in duration, conducted for 5 consecutive days. Spontaneous activity was tested in a Digiscan (Omnitech Inst.) automated activity device measuring 40 X40 cm housed in a dimly lighted chamber. These tests were 16 min in duration, conducted for 5 consecutive days. Most tests were conducted with 15-20 animals per group.

The results, for both the open field and the spontaneous activity tests, showed that at 2 mos of age the behavior of the treated animals was little different from that of the controls. However, at 4 mos of age the treated animals were significantly more active in both test apparatuses. At the intermediate time of 3 mos some differences were noted, but they were not as clear as at 4 mos. These effects seemed to persist, being very pronounced in a small sample of animals tested at 8 mos.

These results indicate that neonatal treatment with chlorimipramine can have enduring effects on animal behavior. However, the effects do not seem to emerge until relatively late in the animal's life.

This research supported by MH40880.

429.7 AN EVALUATION OF CHOLINERGIC INVOLVEMENT IN SELF-STIMULATION OF THE MEDIAL FOREBRAIN BUNDLE. A. M. Robertson and A. Laferrière*. Dept. of Psychology, Univ. of Alberta, Edmonton, Alberta, T6G 2E9.

The effects of cholinergic manipulations on the rewarding consequences of medial forebrain bundle (MFB) stimulation have been described in many studies, usually in negative terms. However, more recent reports suggest that the use of behavioral methods yielding more sensitive measures of the reinforcing efficacy of MFB stimulation could reveal a substantial effect of systematic or intracerebral administration of atropine on MFB reward-related processes (eg. Yeomans et al. Brain Res., 1985, 329, 19; Gratton & Wise, Science, 1985, 227, 4686). In order to extend these findings, lever pressing behavior maintained by MFB stimulation in rats was challenged by cholinergic blockers in two experiments. In the first experiment, reward summation functions were obtained by measuring the variations in response rates to changes in the frequency of cathodal stimulation pulses (expressed as the number of pulses per train of constant duration). Measurements were repeated under vehicle, atropine sulfate (0.5 to 10 mg/kg, IP, or scopolamine (0.5 to 4.0 mg/kg, IP). No significant shifts in the function were seen to occur at any dose of either drug. In the second experiment, the possible presence of a cholinergic component to the directly stimulated reward-related MFB elements was assessed further by measuring current thresholds over a range of stimulating frequencies (25 Hz to 285 Hz). The resultant current-frequency trade off function provides an index of temporal and spatial summation within the substrate (Gallistel et al., Psych. Rev., 1981, 88, 228). The hypothesis that cholinergic blockade results in the inactivation of a proportion of these directly stimulated elements was tested by obtaining current-frequency functions under vehicle or atropine (0.5 to 10 mg/kg, IP). No effects of the drug could be detected on the scale or shape of the functions. Considered together, the two experiments do not support the assertion that cholinergic blockade specifically interferes with MFB processes involved in the production of reinforcing effects. Supported by NSERC Grants A66 to Dr. P.M. Milner and A1636 to Dr. A. Robertson.

429.8 CALCIUM SUPPLEMENTS ELEVATE MOOD IN DEPRESSED HUMAN SUBJECTS. K. Arasteh and M.E. Trulson. Dept. Anatomy, Coll. Med., Texas A&M Univ., College Station, TX 77843.

Many disorders of calcium metabolism are associated with mood changes that are similar to those of affective illness. Furthermore, in such disorders the restoration of calcium levels to normal is accompanied by a change in mood. Cogan et al. (Am. J. Med., 65, 1978, 963), for example, reported a significant improvement in mood for patients with secondary hyperparathyroidism after parathyroidectomy. Also, patients with primary depression exhibit altered levels of calcium in the cerebral spinal fluid and plasma. The recovery from depression, on the other hand, is associated with the return of calcium levels to normal. Since calcium regulates the synthesis and release of neurotransmitters postulated to be involved in the etiology of mood disorders, as well as altering the responsiveness of postsynaptic receptors, altered calcium homeostasis may be involved in mood disorders. In the present study, depressed human subjects, as defined by DSM III criteria, received either calcium (1000 mg) containing vitamin D (600 I.U.) or placebo twice daily for four consecutive weeks. Before and after scores on the Beck Depression Inventory (BDI) indicated that subjects who received calcium plus vitamin D showed a significant elevation in mood as compared to the placebo control group. An analysis of variance (ANOVA) on the data from the 67 participants revealed no significant difference between the calcium and placebo groups on the BDI (calcium group = 16.9 ± 2.1; placebo = 15.8 ± 1.8) upon the first administration, i.e., prior to experimental manipulation. When ANOVA was performed the results of the second administration of the measures the main effect of calcium on the BDI was statistically significant with a lower (i.e., enhanced mood) BDI score in the calcium group (3.7 ± 0.9 vs 14.6 ± 2.4 in the placebo group). The subjects who received calcium plus vitamin D showed significantly higher plasma calcium levels (calcium group = 11.4 ± 0.1 mg/dl; placebo group = 9.6 ± 0.1 mg/dl, $P<0.01$, two-tailed t-test). Thus a strong tendency for calcium plus vitamin D supplements to elevate mood was observed in the present study. We have previously found that calcium does not elevate mood in subjects with normal BDI scores. Therefore, calcium only appears to elevate mood in those who have a more severe form of depression, leaving the non-depressed individuals unaffected. This suggests a role of calcium in the therapy of depression and possibly in its etiology as well.

429.9 HEROIN SELF-ADMINISTRATION IN THE RAT SUPPRESSED BY SCH 23390. S. Nakajima and R. A. Wise Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec, Canada

Operant responding for various reinforcement agents can be suppressed by an i.p. injection of SCH 23390, a selective dopamine D1 receptor antagonist. In the present study, we report a suppression of bar-pressing response reinforced with intravenous heroin infusion.

Hooded rats were implanted with jugular catheters and trained to press a bar in order to infuse heroin hydrochloride, 0.1 mg/kg, in 0.25 ml saline solution. The animals showed a high rate of responding in the first hour (initial loading) followed by a reduced and steady rate in the subsequent 5 hr in each daily session. SCH 23390 was dissolved in methanol first, and then diluted with physiological saline to various final concentrations. Each dose of SCH 23390 was given i.p. 1 hr after the beginning of a self-administration session.

SCH 23390 (0.04-0.08 mg/kg) suppressed heroin self-administration in a dose-related manner. There was no sign of catalepsy or ataxia at these dose levels. At very low dose levels (0.005-0.01 mg/kg), some animals showed an increase in response rate as compared with the rates in no-injection sessions and in a vehicle control session. The dose-related suppression observed in the present study is consistent with the previous findings in operant situations reinforced with food, water, saccharin, and brain stimulation. The increase in response rate at low dose levels is similar to the effect of a low-dose injection of opiate antagonist. It appears that dopamine D1 receptors play an important role in producing the rewarding effect of intravenous heroin injection.

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420.10 SPATIAL ANALYSIS OF LOCOMOTOR BEHAVIOR INDUCED BY INTRACEREBRAL INJECTIONS OF CARBACHOL IN THE RAT. S.M. Brudzynski, R.S. McLachlan and J.P. Girvin. Dept. of Clinical Neurological Sciences, University of Western Ontario, London, Ontario N6A 5C1.

Injections of carbachol (CCh) into the anterior hypothalamic/preoptic area decrease locomotor activity and rearing behavior in rats. We have recently observed that the spatial pattern of animal behavior is also altered by these injections. Thus, in the present study, we have examined time spent by rats in different zones of an activity cage during undisturbed behavior and after intracerebral injections of CCh.

Eighteen Wistar rats were bilaterally implanted with stainless steel cannulae for injections of CCh into the anterior hypothalamic/preoptic area in chronic experiments. Cannulae were inserted into the brain 7-8 mm anterior to the interaural plane according to the Paxinos and Watson stereotaxic atlas. Activity of rats was recorded using 16 infrared lightbeams in a Digiscan Monitor (Omnitech Electronics, Ltd.). The floor of the square recording cage was divided into 9 zones. Locomotor activity and time spent in each of these zones were measured 5 min before and 10 min (2x5 min) after injection of CCh or saline as a control. All the cannulae placements were verified histologically post-mortem. A total of 36 injection sites in the hypothalamic/preoptic area were explored and injected with CCh (1 µg in 0.2 µL) and isotonic saline (0.2 µL) in random order.

Freely behaving rats spent 85.7% (257.0 ± 32.0 s) of the time in all corners (corner time), 14.2% (42.6 ± 32.1 s) in contact with walls between corners, and 0.1% (0.4 ± 1.5 s) in the central zone of the cage during the 5 min before injection. Injection of isotonic saline did not change significantly that pattern. However, injections of CCh into the same brain sites decreased significantly the corner time in the first 5 min post-injection to 61.7% (185.2 ± 63.4 s; t-test, n=36, $p<0.001$), increased time spent between corners to 35.5% (106.6 ± 54.8 s; n=36, $p<0.001$) and in the center of the cage to 2.7% (8.2 ± 13.7 s; n=36; $p<0.002$) as compared to the effects after saline injection. The effect of CCh was dependent on the location of injection site and was not obtained from more rostral areas than 8.5 mm anterior to the interaural plane. Changes in spatial pattern of rat behavior induced by injection of CCh were usually concomitant with a decrease in locomotor activity. Both these behavioral manifestations were reversed by pretreatment with 1.5 µg of atropine.

The results are interpreted in terms of cholinergically-evoked changes in locomotor and emotional components of adaptive behavior.

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429.11 SENSITIZATION OF SELF-STIMULATION AND SPONTANEOUS MOTOR ACTIVITY REDUCTIONS AFTER CHRONIC LOW-DOSE HALOPERIDOL TREATMENT.
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Previous studies have suggested development of tolerance to the neuroleptics' rate-suppressing effects on self-stimulation (SS) behavior (Eichler et al.,Neurosci.,2:848,1976). As these studies employed high doses and examined only response rate (versus threshold) measures, it would seem that tolerance reflects an adaptation to neuroleptic-induced motor impairment similar to that which has been observed for catalepsy. We have previously demonstrated that low dose (0.1 mg/kg) haloperidol (H) treatment produces shifts in both threshold and asymptotic performance dimensions of rate-intensity functions (Lynch & Carey,Neurosci.,12:937,1986). Therefore, VTA-implanted rats (n=5) were given 0.1 mg/kg i.p. H once per day for 10 days in order to track effects of chronic neuroleptic treatment in a dose range which produces subtle behavioral changes indicative of altered reward substrate (H-pre group). Behavioral testing was conducted every 3 days at 1 hr after H. Control rats (n=4) received H after each behavioral test (H-post). Surprizingly, H-pre rats showed an almost complete suppression of SS upon the first H administration, which became even more pronounced by the second test. The acute result was unexpected from our previous observations and it is not clear why inconsistent findings have been reported across groups of animals and test procedures for similar doses of this drug (see e.g.,Lynch & Wise, PBB, 23:777,1985). Tolerance was not observed over repeated administration and testing. In fact, concomitant observations of spontaneous motor activity in an open field revealed sensitization to H's rate-reducing effects on overall behavioral output. Response rates in the H-post group remained at baseline for both behavioral measures. After 10 days of 0.1 mg/kg H, both groups were tested with a series of low doses of H (.025 to 0.1 mg/kg) administered 1 hr prior to testing. Although the groups had similar pharmacological histories, response reductions in the previous H-post group were very small (an approx. 10% reduction in peak SS rates), while H-pre rats exhibited an approx. 50% reduction in peak rates and much lower rates of spontaneous motor activity as well. Decreased asymptotic performance was observed in the absence of reliable threshold shifts that would indicate reinforcement changes. In conclusion, low dose H treatment paired with the SS test chamber contributed to greater response reduction upon subsequent testing with .025 to 0.1 mg/kg H and produced sensitization to reduced spontaneous motor activity that persisted after the chronic treatment with 0.1 H. These environmentally-related neuroleptic effects may be of relevance for interpreting delayed-onset therapeutic and motor effects with clinical usage. Supported by N.I.D.A. Research Service Award 1 F32 DA05291-01A2 DABR-1 to M.R. Lynch.

429.12 CHRONICALLY ISOLATED RATS INTRAVENOUSLY SELF-ADMINISTER MORE COCAINE BUT NOT AMPHETAMINE WHEN COMPARED TO CHRONICALLY AGGREGATED RATS.
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We have been studying the role that environmental factors play in the predisposition to drug abuse. Sensitivity to a number of abused drugs, as measured by a number of behavioral paradigms, is markedly influenced by an early housing manipulation consisting of isolation or aggregation in weanling rats. These conditions, however, do not effect the sensitivity to amphetamine, as measured by place conditioning. In the present study, these findings are extended by examining the propensity of isolated or aggregated rats to intravenously self-administer cocaine or amphetamine.

Male Long Evans rats were obtained at weaning (21 days) and were housed either singly or in groups of 4 for 6 weeks. They were then prepared with chronic, indwelling jugular catheters. Following one week, the rats were introduced to an operant chamber where depression of a lever resulted in a 0.1 ml infusion of cocaine HCl or d-amphetamine SO_4 dissolved in physiological saline. A repeated measure design was used whereby "cocaine" rats were tested with 1.0, 0.5 and 0.1 mg/kg/infusion and "amphetamine" rats were tested with 0.25, 0.125, 0.06, 0.03, 0.015, or 0.004 mg/kg/infusion. Doses were maintained for 2-4 days and were run in descending order. Testing sessions were 3 hours long. Except for this daily 3 hr period, housing conditions were maintained.

The isolated rats self-administered cocaine in a dose-dependent fashion, with maximal rates obtained at the 0.5 mg/kg dose. The grouped rats failed to reliably self-administer this drug regardless of dose. In contrast, rats from both housing conditions self-administered amphetamine, dose-dependently, with maximal rates at the .06 mg/kg dose.

These data support the notion that the reinforcing properties of only some drugs of abuse are influenced by environmental factors. Further, the difference in the effect on cocaine and amphetamine self-administration suggest specific neurochemical changes associated with the manipulation.

429.13 IN VIVO ELECTROCHEMICAL EVIDENCE FOR RELEASE OF DOPAMINE EVOKED BY REWARDING ELECTRICAL STIMULATION.
A. Gratton, G. Gerhardt and B.J. Hoffer, Dept. of Pharmacology, U. of Colorado Hlth. Sci. Ctr., Denver, CO 80262.

There is now considerable psychopharmacological evidence implicating the A10 dopamine (DA) system in brain stimulation reward (BSR). However, more recent psychophysical data indicate that DA fibers are not directly activated with stimulation parameters commonly used to elicit BSR. Rather, it seems that DA may either be efferent to the directly activated substrate of BSR or may in some way modulate the activity of the BSR substrate. While some previous studies have shown medial forebrain bundle (MFB) electrically-evoked releases of DA, the strength of the stimulation used in these studies was often several fold greater than what is required to elicit a rewarding effect. The present study now provides preliminary data suggesting that rewarding levels of MFB stimulation do elicit small but detectable increases of DA release as measured by in vivo high speed chronoamperometry.

Male Long-Evans rats previously trained to lever-press for rewarding stimulation applied to either the MFB or the ventral tegmental area (VTA) were anesthetized with urethane and placed in a stereotaxic apparatus. A Nafion-coated graphite/epoxy capillary (GEC) electrode was then lowered into the various DA terminal fields. Chronoamperometric measurements were obtained by applying square-wave pulses (from -0.2 to +0.45 V) to the GEC at a rate of 5 or 10 Hz. The resulting current flow generated by the oxidation and subsequent reduction of electroactive species at the GEC tip was digitized and integrated over the last 60% of the positive and negative going pulses. Changes in DA concentration were monitored in this manner while a single 500 msec train of 0.1 msec duration cathodal pulses was applied to the MFB or VTA at frequencies in the 20-100 Hz range and at current intensities of 500-800 uamp.

The magnitude of DA releases was positively related to the magnitude (charge) of the stimulation. The duration of DA releases generally outlasted the stimulation by 2-6 secs. Such releases were detected in the nucleus accumbens, caudate nucleus, prefrontal and cingulate cortex. While these data do not prove that the electrically-evoked release of DA is reward-relevant, or if it is elicited by direct or transsynaptic activation of DA neurons, our findings do suggest that rewarding stimulation applied to BSR-relevant MFB and VTA sites is capable of eliciting measurable increases of DA in the extracellular space.

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429.14 DISSOCIATION OF MORPHINE'S DISCRIMINATIVE STIMULUS PROPERTIES FROM ITS MOTIVATIONAL PROPERTIES.
G.M.Martin*, A. Bechara and D.J. van der Kooy. Dept. of Psychology, Memorial University of Newfoundland and Dept. of Anatomy, University of Toronto, Toronto, Canada M5S 1A8

Opiates have both positive reinforcing and aversive motivational properties. In addition, morphine has cueing properties, in that it can serve as a discriminative stimulus. We investigated whether rats use the positive reinforcing or aversive motivational properties of morphine as the perceived property that controls conditional responses. We show that morphine acquires discriminative properties in as little as 5 pairings with a novel solution and an appropriate unconditional stimulus when we use a recently described conditioning procedure (A. Riley et al, Psychon. Soc. Meeting, 27,18, 1986). On alternating days, rats were injected with either 5 mg/kg of morphine or its vehicle 15 min prior to access to a 2.0% vinegar solution. On morphine days an i.p. injection of 130 mg/kg of lithium chloride immediately followed the end of the 15 min exposure to the vinegar. Over the course of 5 morphine trials the rats learned to avoid vinegar on morphine days and to consume vinegar on vehicle days. Control experiments showed that the rats had learned a robust discrimination that could not be attributed to non-specific effects such as the injection regime or the unconditional effects of the morphine or the vinegar solution.

Control over consumption exerted by morphine was mediated specifically by central nervous system opiate receptors. An injection of 0.2 mg/kg of naloxone eliminated the capacity of morphine to control consumption of vinegar, whereas an injection of 10 mg/kg of peripherally-acting methylnaltrexone did not. We conclude that aversive motivational properties of morphine are not necessary cues for the discrimination, since morphine produces its aversive effects (measured with conditioned taste aversions) through peripheral receptors and because methylnaltrexone blocks the aversive effects of morphine. Similarly, the positive reinforcing properties of morphine that support place preferences do not seem responsible for the discriminative control exerted by morphine over vinegar consumption. We removed the neuronal cell bodies of the nucleus tegmenti pedunculopontinus region of the limbic midbrain with a bilateral injection of 0.2 ul of 4.0% ibotenic acid. This lesion prevented 5 mg/kg of morphine from producing place preferences but did not prevent morphine from acting as a cue to control vinegar consumption. These data indicate that the motivational properties of morphine that produce taste aversions and place preferences can be dissociated from the cueing properties of morphine that enable morphine to serve as a discriminative stimulus.

429.15 SEPARATION OF MORPHINE'S INCENTIVE MOTIVATIONAL FROM ITS ESCAPE FROM WITHDRAWL PROPERTIES. A.Bechara and D.van der Kooy. Anatomy Dept.,Faculty of Medicine,Univ.of Toronto,Toronto,Canada M5S 1A8.
In the case of drugs which produce physical dependence such as opiates,it is difficult to differentiate positive motivational or incentive effects from reinforcing effects associated with avoiding or terminating abstinence.In dependent animals most paradigms for assessing the reinforcing effects of drugs measure both properties.Using morphine as a reinforcer and employing a modified place conditioning paradigm,we now demonstrate that these two mechanisms of reinforcement are separable.
Three different conditioning procedures were employed,each comprising two separate groups of drug naive and physically dependent rats(50 mg/kg/day for 14 days).Briefly,procedure B(both) was the standard place conditioning method where conditioning took place in both compartments of the place conditioning apparatus:one environment paired with morphine(2 mg/kg i.p.)and the other environment paired with vehicle injections.Procedures M(morphine only) and W(withdrawl only) were modified place conditioning procedures where conditioning took place in only one compartment of the place conditioning apparatus.That is,procedure M involved morphine pairings(2 mg/kg i.p.)with one environment and vehicle injections in the home cage.Procedure W involved vehicle pairings with one environment and morphine injections(2 mg/kg i.p.)in the home cage.All animals were tested using both compartments of the place conditioning apparatus.Thus, procedure M measured the incentive or positive motivational properties of morphine,whereas procedure W measured the aversiveness associated with morphine abstinence.On the other hand,procedure B measured morphine reinforcement as a combination both of the incentive or positive motivational properties of morphine and of the aversiveness of withdrawl and its relief.
Drug naive animals conditioned according to procedures B and M,showed equally large preferences for the morphine paired environments.Naive rats conditioned according to procedure W did not express any significant place conditioning effects.These results suggest that in the absence of physical dependence (i.e.detectable withdrawl signs),the primary mechanism of opioid reinforcement is related to the incentive or positive motivational effects of opioids.Under these circumstances,opioid abstinence did not have any motivational properties.On the other hand,physically dependent animals(i.e.detectable withdrawl signs)conditioned according to procedure B showed a preference for the morphine paired environment similar to naive rats.However,animals conditioned according to procedure M did not show a significant preference for the morphine paired environment.Most interestingly,physically dependent animals conditioned according to procedure W expressed significant place aversions to the vehicle paired environment.These results suggest that in the presence of physical dependence,the same dose of morphine,over the same route of administration produced positive reinforcing effects similar to naive animals (procedure B).However,in dependent rats,these reinforcing effects were not attributable to the incentive or positive motivational effects of opioids(procedure M).In fact,the primary mechanism underlying the reinforcing effects was avoidance of opioid abstinence(procedure W).The results suggest that the reinforcing mechanisms of morphine in naive and physically dependent animals can be measured separatly and may be independent of each other.

429.16 LATERAL HYPOTHALAMIC NEURONES MODULATE BOTH INTRACRANIAL SELF-STIMULATION OF THE PARABRACHIAL AREA AND THE PALATABILITY OF GUSTATORY STIMULI IN RATS. L.Velley, A.Ferssiwi* and B.Cardo* Lab.Psychophysiologie, Univ.BordeauxI, 33405 Talence Cedex France
The involvement of intrinsic neurones of the lateral hypothalamus (LH) in intracranial self-stimulation (ICSS) of the parabrachial area (PBA) was studied. Rats were bilaterally implanted with self-stimulation electrodes in the PBA and with guide cannulae located above each LH. Rats were tested for ICSS and once responding had stabilized, unilateral ibotenic acid (IBO) lesion of the LH was performed. Eight days later, ICSS was tested in the PBA both ipsilateral and contralateral to the lesion. Intrinsic neurones of the other half of the LH were then lesioned with IBO and ICSS in both sides of the PBA was retested 8, 12, and 30 days later. The unilateral lesion produced a significant decrease of ICSS of PBA ipsilateral to the lesion, without modification of the ICSS of the contralateral PBA. After bilateral lesion, ICSS was greatly reduced bilaterally and the results further suggest that the main effect of the lesion was to decrease ICSS threshold.
In a second experiment, rats with bilateral IBO lesion of the LH and control rats were placed, 13 days after operation on a water-deprivation schedule for 5 consecutive days and were then given the choice between water and one of 5 concentrations of saccharin solution using a 2 bottles procedure. Fluid intake across concentrations generated a preference-aversion curve. The same procedure was used to obtain the aversion curve for 5 increasing concentrations of quinine solution. Lesioned and control rats showed a clear preference-aversion response to saccharin solutions and an aversive response to quinine solutions. However, the highest preference score of the lesioned rats was obtained with a saccharin concentration 3 times higher than the concentration preferred by control rats. In the lesioned rats, the aversion threshold to quinine solutions was obtained with a concentration 5 times higher than the concentration inducing aversion in the control rats. At the end of these experiments, rats used as controls were submitted, in turn, to bilateral lesion of the LH. Although they learned the choice task before the lesion, these newly lesioned rats reproduced the preference-aversion curve to saccharin of lesioned naive rats.
Taken together,our results suggest that: 1) the ICSS in PBA, a relay-station for the gustatory inputs, results from the activation of feedback loops between the LH and the PBA; 2) in the normal rat,the palatability of certain gustatory stimuli is modulated by the intrinsic neurones of the LH.
The quantitative distribution of LH neurones projecting to the PBA was studied by retrograde horseradish peroxidase labelling.
(Supported by INSERM grant 856034).

STRESS, HORMONES AND THE AUTONOMIC NERVOUS SYSTEM II

430.1 PHARMACOLOGICAL AND PHYSICAL ANTAGONISM OF VARIOUS ACTH-INDUCED GROOMING BEHAVIORS: EFFECTS ON BODY TEMPERATURE. D.L. Colbern, D.A. Twombly*, and W.H. Gispen*. Dept. of Physiology and Biophysics, University of Illinois, Chicago, IL 60680.
Rats injected icv with $ACTH_{1-24}$ exhibit a dramatic increase in grooming behavior. Except for their greatly prolonged duration, the behaviors induced by this peptide (face washing, body washing, scratching, & anogenital licking) are qualitatively similar to those observed when rats are placed in a novel environment. Grooming also serves thermoregulatory functions. When exposed to warm environments, rats cool themselves by spreading saliva on their fur; in cold environments, they spread a lipid material which insulates and darkens the fur.
The present experiments examined whether icv injection of ACTH (3ug/3ul) or exposure to a novel environment produce changes in body temperature which could account for the subsequent increase in grooming. Body temperature was also investigated during blockade of grooming by neurotensin (2ug, icv), by naloxone (1mg/kg, sc), or by collars which physically prevented rats from grooming.
Various behaviors (face washing, body washing, scratching, anogenital licking, stretching, yawning, and resting) and core temperature were simultaneously recorded for 70 min beginning 10 min after drug injection. Temperature was recorded with biotelemetry units (Minimitters, Sunriver, OR) surgically implanted into the peritoneal cavity 7 days before the behavioral session. Rats injected with ACTH exhibited significantly more grooming behavior (particularly face and body washing) than did rats injected with saline. Body temperature was increased over basal temperature in both ACTH- and saline-treated rats placed in a novel observation chamber; however, body temperature was significantly higher in ACTH- than saline-treated animals. Body temperature was decreased from baseline in rats treated with either neurotensin or naloxone. Both substances blocked the excessive grooming produced by ACTH. When neurotensin was combined with ACTH, rats became even more hypothermic than with neurotensin alone. On the other hand, the hyperthermic effect of ACTH was enhanced by naloxone, especially during the last part of the session when grooming was most effectively suppressed. In groups fitted with collars, the body temperature of saline-injected rats was increased compared to those injected with ACTH, even though both groups exhibited comparable amounts of resting, movement, or attempted grooming.
The results of these studies suggest that body temperature responds to treatments which affect grooming behavior. However, it is not clear whether changes in body temperature are a cause or consequence of altered grooming, or are merely concomitant with these behaviors.

430.2 OXYTOCIN INDUCTION OF GROOMING IN THE VENTRAL TEGMENTUM, J.A. Stivers*, M.T. Kaltwasser, P.S. Hill*, V.J. Hruby* and J.N. Crawley, (Spon: J.H. Autry) Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20892
Bilateral microinjection of oxytocin (OXY) into the ventral tegmental area (VTA) of rat brain produced a significant increase in grooming behaviors at doses from 100 pg to 400 ng. Sites in the caudal region of the VTA were sensitive to lower doses of OXY than sites in the rostral region of the VTA. The time course of action of OXY in the grooming paradigm indicated onset beginning immediately after injection, and termination at 60-75 minutes after injection. Comparison of OXY-induced grooming in male, female, and ovarectomized, estrogen-treated female rats showed no differences in potency for OXY among these groups, suggesting that the grooming effects of OXY are not regulated by sex steroids. Analysis of locomotor activity in rats microinjected with OXY 200 ng bilaterally into the caudal VTA revealed that OXY had no effect on ambulatory locomotion, suggesting that this peptide may activate neurons within the VTA which mediate grooming but not locomotion. The OXY receptor antagonist, [Pen^1, pMePhe^2, Thr^4, Orn^8]-OT, blocked OXY-induced grooming when both were simultaneously microinjected into the VTA. The dopamine D-2 receptor antagonist, haloperidol, and the D-1 receptor antagonist, SCH 23390, when microinjected into the VTA five minutes before microinjection of OXY into the VTA, did not block OXY-induced grooming, suggesting that OXY is working through its own receptor rather than through a dopamine autoreceptor on the VTA neurons. Systemic pretreatment with haloperidol and SCH 23390 effectively blocked grooming induced by OXY in the VTA. These data indicate that OXY is directly stimulating OXY receptors on VTA neurons to release dopamine at postsynaptic sites regulating grooming behaviors.

430.3 GROOMING AND GNAWING DURING HALOPERIDOL-INDUCED CATALEPSY. C. Van Hartesveldt. Psychology Department and Center for Neurobiological Sciences, U. Florida, Gainesville, FL 32610.

Measures of haloperidol-induced catalepsy such as clinging to a horizontal bar or to a vertical wire grid increase in a dose-dependent manner. In this experiment grooming and gnawing, behaviors not usually associated with catalepsy, were also found to increase significantly with increasing doses of haloperidol during catalepsy tests.

Male Long-Evans hooded rats were each injected SC with each of four doses of haloperidol in a randomized order: 0.05, 0.1, 0.2, and 0.4 mg/kg. After injection each rat was tested every 30 minutes for 2 hours for both horizontal bar clinging and vertical wire grid clinging. For the former, each rat was placed with its forelimbs on a horizontal bar, and duration of bar clinging without moving a forelimb was measured in two trials with a 30 second inter-trial interval. After another 30 second interval, the rat was placed on a near-vertical wire grid, and duration of clinging without moving a foot was measured in two trials with a 30 second inter-trial interval. The cutoff for each clinging trial was 2 minutes. During these tests the occurrence of grooming and gnawing were also noted.

As expected, both horizontal bar clinging and vertical grid clinging increased significantly as the dose of haloperidol increased. There was also an unexpected increase in both gnawing and grooming during the catalepsy tests. After the highest dose, rats frequently groomed while on the horizontal bar, and gnawed on the vertical wire grid. Number of rats gnawing or grooming increased significantly in a dose-dependent manner, as did number of grooming episodes.

These findings were unexpected since in some situations both gnawing and grooming can be enhanced by dopamine agonists, and both can be blocked by dopamine antagonists such as haloperidol. Since these behaviors were not observed in the home cage between trials, they may be due to an interaction between haloperidol and the procedures of catalepsy testing.

430.4 BOMBESIN-INDUCED GROOMING IN THE GOLDEN HAMSTER: NEUROPHARMACOLOGICAL BLOCKADE. P.J. Kulkosky, M.A. Foderaro*, G.W. Glazner*, V.I. Niichel* and P. Schnur. Dept. of Psychology, Univ. of Southern Colorado, Pueblo, CO 81001.

Bombesin (BBS) is an anuran-derived tetradecapeptide with structural counterparts in mammalian brain and gut that are viewed as candidate neuromodulators. Cerebral ventricular application of BBS and BBS-like peptides robustly elicits a syndrome of excessive grooming and scratching behaviors in the mouse, rat, guinea pig, rabbit, and rhesus monkey, but not in the pigeon (cf. Cowan, Life Sci., 37:135, 1985). Recently, bombesin-induced excessive grooming was briefly noted in food and water-deprived golden hamsters by Miceli and Malsbury (Behav. Neurosci., 99:1192, 1985). We characterized the following groomological properties of centrally-administered BBS in the golden hamster: dose-response, time-course, response topography, and neuropharmacological sensitivity. Male and female golden Syrian hamsters received intracerebroventricular (icv) injections of either artificial cerebrospinal fluid (CSF) or bombesin (0.001-1.0 µg/5 µL) and grooming behavior was observed and categorized at tone-cued 0.6 sec intervals each minute for 60 min after injection. The threshold dose of BBS-induced excessive grooming is 0.01 µg in the hamster. Grooming persisted throughout the observation period at doses of 0.1 and 1.0 µg. At all effective doses, forepaw and mouth grooming behaviors were increased more than hindleg scratching behaviors. To assess neuropharmacological sensitivity of this effect, hamsters received either intraperitoneal saline or 5.0 mg/kg haloperidol, 1.0 mg/kg naloxone, 20.0 mg/kg pentazocine, or 0.001, 0.01, 0.1 or 1.0 mg/kg scopolamine, 15 min prior to icv CSF or 0.1 µg BBS. Naloxone and pentazocine significantly and slightly reduced BBS-induced grooming, but did not affect basal level of grooming. Scopolamine abolished BBS-induced grooming at doses of 0.1 and 1.0 mg/kg, but did not abolish basal level of grooming. The blockade of BBS-induced grooming by peripheral scopolamine is consistent with observations made in the rat by Merali, Johnston and Kateb (Abst. N.Y.A.S. Conf. Neural Mech. Biol. Significance Grooming Behav., P 11, 1986), and suggests consideration of the use of anticholinergics in treatment of pruritis associated with BBS-secreting lung tumors. The results allow a characterization of BBS-induced grooming in the golden hamster as potent and persistent, involving primarily forepaw and mouth grooming, influenced slightly by opioid mechanisms, and dependent on muscarinic, cholinergic neural activity. These results confirm a cross-species generality of BBS-induced grooming, but also indicate species-specificity of response topography and neuropharmacological sensitivities of this neuropeptide effect on skin surface maintenance behaviors. (Supported by NIH Grant No. RR-08197).

430.5 ENHANCED NOVELTY-INDUCED GROOMING RESPONSE OF THE DIABETIC RAT MAY BE ASSOCIATED WITH D-1 RECEPTOR SUPERSENSITIVITY. Z. Merali & Q. Ahmad. Psychology & Pharmacology. University of Ottawa, 275 Nicholas St., Ottawa, Ont. Canada. K1N 6N5

In the spontaneously diabetic Wistar BB-Rat (SDR), we have observed an attenuated behavioural response to d-amphetamine, apomorphine and circadian cues. However, the grooming response to a novel environment was significantly enhanced in the SDR as compared to the non-diabetic animals. Under a more stressful condition (two serial i.p. saline injections), the non-diabetics and SDR groomed equally as much. Since the dopamine D-1 receptors have been implicated in the expression of grooming, we wanted to explore whether the sensitivity of the D-1 receptors was increased in the SDR. Thus SDR rats (maintained on insulin therapy) and appropriate control rats were administered a selective D-1 receptor agonist (SKF 38393, 0.0-10.0 mg/kg; i.p.), and the grooming monitored for 1 hr. A time-course analyses revealed that most of the grooming occurred within the first 15 min following treatment. Hence all the data were analyzed for that duration only. In control rats (genetically distinct group), SKF 38393 induced grooming in a dose-dependent manner; at the 5 and 10 mg/kg doses, control rats groomed 3x and 6x as much, respectively, as at the 0.1 mg/kg dose. In the SDR rats, SKF 38393 stimulated grooming at 0.5 and 1.0 mg/kg but not at 0.1, 2.0, 5.0 or 10.0 mg/kg doses, producing a bell-shaped dose-response curve. It is of interest to note that the SDR rats groomed more at 0.5 and 1.0 mg/kg dose than the controls at 5.0 and 10.0 mg/kg doses, respectively. Therefore, the diabetic rats appeared to be at least an order of magnitude more sensitive to the SKF 38393 induced grooming. The genetic littermates of the SDR, that failed to develop the symptoms of diabetes, constituted the non-diabetic group. These rats were slightly more sensitive to the grooming effects of SKF 38393 than the genetically distinct controls but not as sensitive as the SDR. Thus our data demonstrates that the SDR may have supersensitive D-1 receptors. This may be a compensatory response to the contended diminution in the intrasynaptic release of dopamine in the diabetic rats. Preliminary results also indicate that treatment of control or SDR with a D-1 antagonist (SCH 23390; 30 ug/kg) blocked the induction of novelty-induced grooming. Therefore, our data also suggests that D-1 receptors may be involved in the expression of novelty-induced grooming.

430.6 CODEINE AND MORPHINE EFFECTS ON GROOMING. A.M.Danks*, W.H.Gispen*#, J.Brakkee*#, K.Shefman*#, and R.L.Isaacson. Center for Neurobehavioral Sciences, Dept. of Psychology, University Center at Binghamton, Binghamton, NY 13901, and #Institute of Molecular Biology, University of Utrecht, Utrecht, Netherlands.

Previous studies have shown that the codeinone RX 336-M injected peripherally can induce excessive grooming in rats. The amount of grooming elicited by the drug was both age- and sex-dependent. No excessive drug-induced grooming was observed in rats of either sex at 60 days of age or older. The present studies were undertaken to determine whether codeine, itself, administered peripherally, would have similar effects to the codeinone. Because of the chemical similarity between codeine and morphine, we also examined grooming elicited by the peripheral injection of morphine as compared to that elicited by codeine. In addition, we studied grooming behaviors elicited by the central administration of codeine.

For the peripheral injections, a dose of 3.0 mg/kg (free base) of codeine or morphine was used. Intraventricular (icv) dosages were 0.3 ug/ul or 1.0 ug/ul. In each study, drug-treated animals were compared to animals injected with an equal volume of saline. The icv administration of the drug and the scoring of excessive grooming were accomplished using methods commonly employed in this laboratory.

Peripheral injections of codeine produced an enhancement of grooming over saline-treated animals. The effects were greater in younger than in older animals. However, in contrast to the results obtained with RX 336-M, a significant enhancement of grooming was also found after peripheral administration in the adults. Peripherally administered morphine produced a different pattern of grooming than that of codeine. It suppressed grooming during the first 30 minutes of observation, and accentuated grooming thereafter, the greatest amount occurring 60-75 minutes after the start of observation. Surprisingly, central administration of codeine, at either of the dosages used, had no effect on grooming. This stands in contrast to studies that have shown that icv administration of morphine produces excessive grooming and does so in the same manner as it does after peripheral administration. Based on these findings, therefore, it appears that codeine and morphine induce excessive grooming by different mechanisms.

430.7 STRESS-INDUCED OPIOID ANALGESIA IN DEER MICE: SEX AND POPULATION DIFFERENCES. M. Kavaliers and D.G.L. Innes*, Division of Oral Biology, The University of Western Ontario, London, Ontario, Canada.

Deer mice, Peromyscus maniculatus, provide an opportunity to assess the effects of natural selection on opioid activity and behavioral responses to stress. Deer mice from various habitats and geographical areas differ in a variety of behavioral, ecological and morphological characteristics. Of particular evolutionary interest are the differences between island and mainland populations of deer mice and other small rodents. In the present study, we describe the restraint-stress induced opioid analgesic and locomotory responses of four different populations of male and female deer mice; Peromyscus maniculatus artemisiae and P. m. nebrasciensis from two mainland regions, and P. m. angustus and P. M. trianqularis from two small coastal islands. All of the mice displayed immobilization-induced analgesia which was blocked by peripheral administrations of the prototypical mu opiate antagonist naloxone (1.0 mg/kg). In all of the populations, males displayed significantly greater levels of analgesia than females. In addition, the levels of analgesia were significantly greater in the insular than in the mainland male and female mice. Restraint also induced significant increases in the locomotor activity of the mainland deer mice, while significantly decreasing the activity of the insular animals. Males displayed significantly greater stress-induced changes in locomotor activity than females. The stress-induced increases in activity of the mainland populations were blocked by the specific delta opiate antagonist, ICI 154,129 (10 mg/kg), while the decreases in activity of the insular animals were inhibited by naloxone (1.0 mg/kg). These findings demonstrate that there are marked sex and population differences in the responses to stress and opioid activation in deer mice. These results suggest that behavioral differences between island and mainland populations may, in part, be related to differences in endogenous opioid activity.

430.8 BODY ROTATION-INDUCED OPIOID ANALGESIA IN DEER MICE: POPULATION DIFFERENCES AND POSSIBLE ECOLOGICAL CORRELATES. D.G. Innes*, M. Kavaliers and K.-P. Ossenkopp, (SPON: R.R. Shivers), Div. of Oral Biology and Dept. of Psychology, University of Western Ontario, Canada.

Recently, various populations of deer mice, Peromyscus maniculatus, have been used to examine the ecological relevance and adaptiveness of stress-induced opioid activation and analgesia. Body rotation, which has been related to vestibular stimulation and motion sickness, is a commonly used stressor that is of both basic and applied interest. In the present study we examined the effects of continuous and intermittent body rotation on the nociceptive responses of island and mainland derived population of deer mice, P.m. augustus and P.m. artemisiae, respectively. These populations were chosen because their natural habitats differ, the island populations being found to habitat in which they are more likely to experience complex vestibular stimulation.

Various groups of island and mainland populations of male mice were exposed to either continuous (79 rpm) or intermittent (5, 30 sec on, off) body rotation for varying periods of time (5-30 min). At specific times (0-60 min) after the rotation the foot-licking response to a surface held at 50 C was determined for individual mice. Mice exposed to either continuous or intermittent rotation were analgesic, displaying significantly greater response latencies than sham-rotated or control animals. Pre-treatment with naloxone (1.0 mg/kg) blocked both the continuous and intermittent rotation-induced analgesia. In addition, there was a greater level of analgesia after exposure to the intermitten than the continuous rotation. In all cases, the insular mice displayed significantly greater levels of rotation-induced analgesia than did the mainland population animals. The results indicate that the degree of body rotation-induced analgesia differs between population in a manner consistent with their likelihood to be exposed to complex vestibular stimulation in the wild. These findings may have implications for the evolutionary basis of motion sickness.

430.9 WHEEL RUNNING AND GASTRIC ULCERATION IN THE RAT FOLLOWING PREADAPTATION TO RESTRICTED FEEDING. N.S. Morrow and S.W. Kiefer. Dept. of Psychology, Kansas State University, Manhattan, KS 66506.

When a rat is housed in an activity wheel and is restricted to eating for 1 hr each day, it runs progressively more, develops ulcers, and results in the demise of the animal. The relationship between preadaptation to restricted feeding, gastric ulceration, and elevated running is not clear as conflicting results have been reported (see Pare et al., Physiol. Behav., 1985, 34, 423-429). In the present study the effect of preadaptation to restricted feeding on wheel running and ulceration was examined by preadapting animals for either 0, 15, 25, or 35 days.

Fifty-six naive rats were housed in activity wheels for 5 days to record baseline measures of food intake, water intake, body weight, and running activity (recorded across 3 daily time periods). Rats were then matched on mean baseline activity and divided into 4 experimental (n=7 each) and 4 control groups (n=7 each). Groups were preadapted to a 1 hr restricted feeding schedule for either 0, 15, 25, or 35 days; experimental groups re-entered the activity wheels following preadaptation experience, whereas control groups remained in individual home cages. Rats were fed between 7:30 PM and 8:30 PM. Food intake, water intake, body weight, and running activity was recorded on a daily basis. Activity rats (and home-cage rats) were sacrificed at the end of the 18 day testing period or whenever an activity animal became moribund. Following sacrifice, the stomach was excised and later scored for gastric ulceration.

Regardless of preadaptation experience, total daily running increased, with the greatest change occurring during the time period (11:10 AM to 6:30 PM) preceding the feeding hour. Running during the remaining two times did not change significantly. Rats with previous preadaptation experience increased running sooner than rats not preadapted. Results further indicated that rats preadapted for 15 days had significantly fewer ulcers ($p < .05$) than the nonadapted group. Rats preadapted for 25 and 35 days also had less ulcers than rats not adapted ($.05 < p < .12$). Increasing preadaptation experience directly increased the number of rats which survived. Results are discussed in terms of the gastric status of the rat as an impetus for excessive wheel running.

430.10 EFFECT OF DIETARY CALCIUM ON STRESS-INDUCED CATECHOLAMINE RELEASE AND SENSITIVITY IN THE SPONTANEOUSLY HYPERTENSIVE RAT. K.E. Scrogin,* D.G. Hatton,* J.A. Metz,* M.S. Muntzel,* D.A. McCarron* (SPON: DENNIS R. TRUNE). Dept. of Med. Psychology, Ore. Health Sciences Univ., Portland, OR 97201.

Modifications in dietary calcium have been shown to alter blood pressure in the spontaneously hypertensive rat (SHR). Supplemental calcium attenuates the development of hypertension in these rats while calcium deprivation potentiates the rise in blood pressure. Similar results have been seen with respect to blood pressure reactivity. A low calcium diet causes greater pressor responses to stress while high calcium reduces reactivity.

This study was designed to determine whether increased catecholaminergic activity or increased sensitivity to catecholamines might be responsible for differences across diets. Both the SHR and its normotensive control strain the Wistar-Kyoto (WKY) were maintained on low (0.1%), medium (1.0%) and high (2.0%) calcium diets and assessed for stress-induced increases in circulating catecholamines as well as pressor responses to catecholamine infusion. Results of this study demonstrated that SHRs had significantly higher blood pressures than their normotensive control strain, the Wistar-Kyoto (WKY). Furthermore, SHRs on a low calcium diet demonstrated higher blood pressures during both handling and restraint stress than did SHRs maintained on a high calcium diet ($p<.05$). In contrast, unstressed SHRs showed no difference in blood pressure across dietary conditions. No dietary effects were seen in the WKY. Analysis of stress-induced release of circulating catecholamines demonstrated no differences across diets in the SHR. However, determinations of catecholamine sensitivity in a dose response study indicated that SHRs maintained on a low calcium diet exhibited significantly larger increases in blood pressure to norepinephrine infusion ($p<.05$). Thus, it seems likely that differences in blood pressure reactivity across diets observed in the SHR are a consequence of altered post-synaptic sensitivity and not an increase in circulating catecholamines.

430.11 THE EFFECT OF LESIONS OF THE AMYGDALOID CENTRAL NUCLEUS ON FEAR INDUCED ARRHYTHMIAS IN THE RABBIT RECEIVING DIGITALIS.
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A variety of risk factors (e.g., coronary artery disease, digitalis) have been identified in the genesis of potentially lethal arrhythmias, due in part to their ability to predispose the heart to electrical instability. Furthermore, clinical and animal observations suggest that a prevalent condition for the occurrence of such arrhythmias is the superimposition of an emotional state upon an existing risk factor(s) (Lown et al., 1979; Natelson, 1979).

Recently, we demonstrated in rabbit that (a) a fear-arousing conditioned stimulus (CS) during retention of Pavlovian fear conditioning induced arrhythmias in the presence of digitalis, and (b) vagal activation in response to the CS contributed to the generation of these arrhythmias (Markgraf et al., 1985). Furthermore, we demonstrated that electrical stimulation of the amygdaloid central nucleus (ACE), a structure implicated in the expression of vagus-mediated conditioned bradycardia in the rabbit (Kapp et al., 1984), during digitalis infusion induced vagus-mediated arrhythmias similar in topography to those observed to the CS.

Since lesions of the ACE attenuate the retention of vagus-mediated, conditioned bradycardia in response to a fear arousing CS (Gentile et al., 1986), we sought to determine if ACE lesions would attenuate CS-induced arrhythmias in rabbits receiving digitalis during retention. Twenty New Zealand rabbits received 20 Pavlovian fear conditioning trials in which a 5.0 sec tone CS was paired with a 0.5 sec, 2.0 mA shock. Twenty four hours later, five rabbits received bilateral radio frequency lesions aimed at the ACE via electrodes implanted prior to conditioning. Nine rabbits served as sham operated and six as unoperated controls. Twenty-four hours following the lesion procedure, a retention test was given during which digitalis was infused i.v. (110 ug/kg), and 20 CS alone trials were presented. The results demonstrated a significant increase in the frequency of arrhythmic episodes during the CS when compared with pre-CS baseline frequencies for both sham and unoperated controls ($p's < 0.05$). No such increase in the frequency of arrhythmic episodes during the CS was observed in the ACE lesion group. The frequency of arrhythmic episodes during the pre-CS period was unaffected by lesions of the ACE.

The results compliment our previous results demonstrating that ACE stimulation induces arrhythmias and suggest that the ACE contributes to the generation of arrhythmias in response to a fear arousing CS in the myocardium predisposed to electrical instability with digitalis. Supported by a grant from the American Heart Association, Vermont Affiliate.

430.12 PHYSIOLOGICAL AND BEHAVIORAL CORRELATES OF SCHEDULE-INDUCED POLYDIPSIA IN RATS.
A. Tazi*, C. Terlouw*+, A. Colas*, R. Dantzer*, G.F. Koob° and M. Le Moal (SPON: D. Beaubaton). INSERM U-259, rue Camille Saint Saëns 33077 Bordeaux Cedex, France;°Scripps Clin. and Res. Fdn.,La Jolla, CA, USA;+Dept. Physiol., Univ. Gröningen, The Netherlands.

Recent research has emphasized the existence of individual differences in the predisposition to develop schedule-induced polydipsia (SIP) in rats exposed to intermittent food (Mittleman and Valenstein,1986; Tazi et al.,1987). In the present experiments, we have further explored the physiological and behavioral characteristics of SIP-positive and SIP-negative rats.

In experiment 1, rats implanted with a chronic venous catheter were submitted to daily sessions of intermittent food. SIP-positive rats exhibited a decrease in plasma corticosterone levels. In contrast SIP-negative rats displayed a gradual increase in plasma corticosterone levels. There was, however, no difference in plasma prolactin levels that increased uniformely in the two groups. When all the rats were tested without water, SIP-positive rats displayed an increase in corticosterone levels similar to that observed in SIP-negative rats.

In experiment 2, rats previously tested for SIP were submitted to conditioned avoidance learning in a 2-way shuttle box. SIP-positive rats learned faster than SIP-negative rats. Moreover, a positive correlation was observed between the amount of water consumed and the number of avoidance responses ($rs = 0.56$; $p < 0.05$).

Experiment 3 extended this observation to a social situation where SIP-positive and SIP-negative rats were placed as intruders into the home cage of a resident rat that had been trained to reliably attack the intruder. Videotape observation of the behavior of the intruder during a 10 min session revealed a tendency for SIP-positive rats to display less submissive-defensive behavior than SIP-negative rats in response to the resident's attacks.

These data demonstrate that differences in the predisposition to develop adjunctive behavior are accompanied by various physiological and behavioral differences. Whether such differences preexist to the SIP experience or are the result of repeated episodes of excessive drinking is not yet determined.

430.13 METABOLIC CORRELATES OF SCHEDULE-INDUCED POLYDIPSIA.
G. Mittleman*, J. Brener* and T.W. Robbins*, (SPON: C.D. BLAHA) Departments of Psychology, The University of British Columbia, Vancouver, B.C., Canada, The University of Hull, Hull, UK, and The University of Cambridge, Cambridge, UK.

Schedule-induced polydipsia (SIP) is an example of the larger category of adjunctive behaviors that occur in situations where a strongly motivated appetitive or consummatory behavior is interrupted or prevented. It has been suggested that SIP as well as other adjunctive behaviors are adaptive as they serve to maintain an animal in a situation that is favorable to its survival. Consistent with this hypothesis is the suggestion that SIP is a coping response that can reduce the heightened arousal caused by an intermittent schedule of food presentation. Hungry animals exposed to intermittent presentations of small amounts of food show increased arousal as measured by either endocrine or behavioral indices (Brett, L.P. and Levine, S., JCPP, 93:946, 1979; Kileen, P., Hanson, S.J. and Osbourne, S.R., Psychol. Rev., 85:571, 1978). In support of the coping hypothesis, pituitary-adrenal activity is significantly reduced in animals showing SIP. However, as Lacey and Lacey (J. Pers. Soc. Psych., 30:1, 1974) have shown, physiological indices of arousal are not always correlated with each other. The interpretation of SIP as a coping response which regulates arousal is therefore critically dependent on which physiological measure is used. In this experiment, rats food deprived to 85% of their baseline weight, were given 15 daily tests for schedule-induced drinking. A food pellet (90 mg) was dispensed every 60 seconds of the 30 minute tests. In addition to recording licking, general activity and entries into the food tray, oxygen consumption (VO_2) and heart rate (HR) were continuously monitored. By day 10, 73% of the animals had developed significant polydipsia. There were systematic changes in VO_2, HR, general activity and tray entries that were time-locked to the point of food delivery. Comparisons between drinking and non-drinking rats and between trials when drinking occurred and when it did not occur indicated that drinking was consistently associated with increased oxygen consumption, increased activity, but decreased heart-rate. These results are mainly consistent with the interpretation that SIP provides a motor outlet for the expression of excessive levels of mobilized energy. However, further characterization of this relationship will require precise measurement of temporal variations in VO_2 and licking. The findings emphasize the importance of taking multiple behavioral and physiological indices of arousal and question the unitary nature of this construct.

430.14 HDL-CHOLESTEROL ELEVATION BY ACUTE STRESS IN THE RAT.
G.D. Coover and R.F. Murry*. Dept. of Psychology, Northern Illinois University, DeKalb, IL 60115.

We previously reported (Coover et al., Soc. for Neurosci. Abst., 1986, 12, 530) that 90-day-old rats exhibit a suppression of the blood level of cholesterol associated with the lower density lipoproteins (lDL-c) in response to the stress of a jugular blood sampling (ether anesthesia and withdrawal of 1.5 ml blood from the surgically exposed jugular vein) followed 15 min later by a footshock (FS) session of 20 min or 1 hr duration. High density lipoprotein cholesterol (HDL-c) tended to increase and then decrease, and in fact decreased significantly in older (165 days) rats by 3 hr following the 1-hr FS session. However, neither the jugular blood sampling, nor the 1-hr FS session, alone were found to cause such short-term changes in blood cholesterol.

In the research presented here, 90-day-old male rats were subjected to a jugular blood sample on Day 1, and then on Day 3 were given an FS session involving 2-sec footshocks on a variable-interval 2-min schedule at an intensity of 2.4 ma no-load setting on BRS-Foringer SGS-003 shocker/scramblers (equivalent to .7-1.0 ma on most shockers), followed by a final blood sample. If no footshock were given on Day 3 (FS session duration = 0), the blood showed, compared to Day 1, an 8.7% increase in total serum cholesterol (from 73.8 to 80.2 mg/dl, $t(16) = 7.1$, $p < .001$) that was associated with an 8.6% increase in HDL-c (from 48.7 to 52.9 mg/dl, $t(16) = 4.84$, $p < .001$). From this elevated baseline on Day 3, total serum cholesterol levels were not significantly changed by footshock stress. In contrast, HDL-c was raised over the higher baseline of Day 3 by an additional 10.1% with a 20-min FS session, $t(22) = 3.03$, $p < .01$, and 12.9% with a 40-min FS session, $t(22) = 4.34$, $p < .001$.

With FS sessions of 80, 160, or 320 min, HDL-c levels, like total serum cholesterol, were not different from the Day 3 baseline. However, in additional comparison groups, it was found that the combined stress of a jugular blood sampling plus the 160-min FS session beginning 15 min later caused significant drops in total serum cholesterol by the end of the session; this occurred when the rats had also been blood sampled on Day 1 (from 84.3 to 72.3 mg/dl, $t(6) = 6.8$, $p < .01$), as well as when they had not (from 73.7 to 66.8, $t(10) = 3.67$, $p < .01$). In the former condition, where baseline HDL-c is elevated over Day 1, a 6.7% decline in HDL-c occurred (from 52.0 to 48.5 mg/dl, $t(6) = 3.14$, $p < .025$) as well as a 26% decline in lDL-c (from 32.3 to 23.8 mg/dl, $t(6) = 5.55$, $p < .001$).

HDL-c levels can be elevated by the relatively mild stress of a jugular blood sample or a brief footshock session. However, the combination of the two stressors seems necessary and sufficient to lower lDL-c, and it can lower HDL-c under extreme conditions.

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430.15 GENETIC ANALYSIS OF SALT APPETITE AND BLOOD PRESSURE IN SHR, WKY, AND HYBRIDS. B.G.Yongue and M.M. Myers. Columbia University and New York State Psychiatric Institute. New York, NY 10032

Spontaneously hypertensive rats (SHR) not only have high blood pressure, but also express an elevated sodium appetite compared to Wistar-Kyoto rats (WKY). We examined whether these traits were genetically coupled to one another. Establishing a linkage between traits first requires a demonstration that the traits cosegregate in hybrid matings of the parent strains and their offspring. In homozygous strains all genes are "fixed" whether they are related to the trait upon which the strain was selected or not. Thus, SHR and WKY differ in many traits that are not related to blood pressure. In cosegregation analysis, SHR and WKY are cross-mated to generate the F1 offspring which are interbred to produce the F2 generation. SHR, WKY, and F1's are homozygous for high, normal, and intermediate blood pressure, respectively, whereas the F2's are heterozygous for the full range of genetically determined blood pressure. If traits are genetically linked they must cosegregate, resulting in a strong correlation in the F2 population. If two traits are not linked, they will segregate randomly with respect to one another in the F2 generation, resulting in no correlation.

We examined the relationship of blood pressure and salt appetite in male SHR, WKY, F1, and F2 rats, 90-140 days of age. Blood pressure was measured prior to salt appetite testing using the indirect tail-cuff method. Subjects had ad lib access (for 6 days) to water, 1.5% NaCl, and Teklad low sodium chow to ensure that saline consumption represented sodium appetite (need) as well as discretionary sodium intake. Fluid intake was measured daily.

The mean blood pressure of SHR was significantly greater than all other groups, and the WKY pressure was significantly lower than all others. As expected, the F1 and F2 groups were near mid-parent values, and did not differ from each other. However, 1.5% NaCl intake did not follow an additive genetic model as does blood pressure. There was a big difference in saline intake between SHR and WKY, but the F1 and F2 rats behaved similar to WKY rats rather than intermediate between WKY and SHR. Moreover, there was no correlation between blood pressure and salt intake in the F2's, indicating there is not a common genetic basis for the two traits.

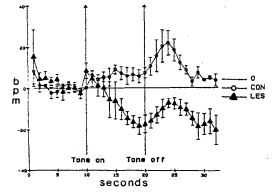
Another lab also reported recently that salt appetite and blood pressure are not related in F2 rats (Harrap, Hypertension, 1986). However, that study, which used hypotonic (~.22%) saline, found that F1 and F2 salt appetite was SHR-like rather than WKY-like. Thus, it is possible that neither the WKY nor the SHR salt appetite phenotype is dominant. Instead, it appears that phenotypic expression of salt appetite in F1 and F2 rats may be partially determined by the concentration of the saline solution.

430.16 EFFECTS OF RAT MEDIAL FRONTAL CORTEX LESIONS ON CONDITIONED EMOTIONAL RESPONSES. R.J. Frysztak* and E.J. Neafsey. Department of Anatomy, Loyola University Medical Center, Maywood, IL 60153

The rat medial frontal cortex (MFC) projects directly to the vagal solitary nucleus (Terreberry & Neafsey, BR 278. 1983), and this pathway appears to influence heart rate (HR), blood pressure (BP), and respiration in both the awake and anesthetized rat. We have begun studying the effects of MFC lesions on the HR and BP components of the conditioned emotional response (CER) in rats.

Male Sprague-Dawley rats were used; four sustained bilateral MFC lesions (bregma to frontal pole, surface to 5 mm below surface, midline to 2 mm lateral) via aspiration, while controls consisted of 3 sham operated and one normal rat. All rats were conditioned two weeks after the initial surgery. The conditioned stimulus was a 10 second, 800 hz tone, and the unconditioned stimulus was a 2 mamp (<1 sec duration) footshock delivered through a metal grid floor. The conditioning process consisted of 10 tones for orientation, followed by 30 tones paired immediately with the footshock. After conditioning, the animal was anesthetized and femoral artery and vein cannulas were implanted along with EKG leads. The following day, BP and HR were recorded during CER trials using a chart recorder and a microcomputer. Data were recorded for a 35 second period, with the tone occurring at 10 seconds; the 5 seconds prior to the tone served as the baseline for each trial. Three CER trials were averaged second by second for each animal. These individual animal averages were then averaged into a composite average for each of the two groups (N=4 in each group).

The BP responses in controls and lesioned rats were similar. Both responded initially to the tone with an increase of 7-10mm Hg. The HR responses between the two groups contrasted sharply (see figure; error bars indicate ±1 SEM). The controls responded to the tone with a slight increase initially (+5 bpm) followed by a large increase coinciding with the time of the expected shock (+20 bpm). The lesion group responded with a decrease in HR, with the maximum drop (-20 bpm) occurring near the time of the expected shock. This pattern of BP + HR responses in the lesioned group resembles that of the normal baroreceptor reflex, suggesting a possible mechanism. During stress in normal animals, the baroreceptor reflex may be inhibited by the MFC, and HR and BP both increase. By lesioning the MFC, the cortical terminals in the NTS which may inhibit the baroreceptor reflex are lost, thus allowing this reflex to operate during the CER. (Supported by a Pott's fund grant from Loyola University.)



430.17 REGIONAL VARIATIONS IN STRESSOR PROVOKED ALTERATIONS OF INTRACRANIAL SELF-STIMULATION FROM THE VENTRAL TEGMENTAL AREA. Marilyn Kasian*, Robert M. Zacharko and Hymie Anisman. Dept. of Psychology, Carleton University, Ottawa, Ontario, CANADA K1S 5B6.

Uncontrollable stressors alter the rewarding value of intracranial self-stimulation (ICSS) from mesolimbic sites, without affecting responding for brain stimulation from the nigrostriatal system. In particular, marked reductions in rates of responding for previously rewarding brain stimulation and changes in current intensity functions have been noted from both the nucleus accumbens and the ventral tegmental area (VTA), while ICSS from the substantia nigra is unaffected by stressor application. These brain region specific effects of uncontrollable stressors on ICSS performance appear to parallel the stressor induced variations of dopamine (DA) activity, in that stressors were found to affect mesolimbic and mesocortical DA turnover, without affecting DA activity in the substantia nigra. We now report that the effects of uncontrollable stressors on ICSS in several strains of mice (CD-1, C57BL/6J, BALB/cByJ) vary across different portions of the VTA. Furthermore, the effects of desmethylimipramine (DMI) on stressor induced responding from these regions are distinguishable from those previously noted from the nucleus accumbens. Footshock was found to reduce ICSS from the lateral and ventral VTA, but actually increased responding from the dorsal VTA. Moreover, unlike the effects observed from the nucleus accumbens, where repeated DMI antagonized the behavioral suppression induced by a stressor, DMI treatment was generally ineffective in reversing ICSS alterations from the VTA. In accordance with the anatomical descriptions provided by Lindvall and Bjorklund (1984), HRP tracings were determined for the ventral, lateral and dorsal regions of the VTA. Our results suggest that the pathways ascending from these relatively discrete areas of the VTA are differentially sensitive to uncontrollable stressor application. This differential sensitivity is in turn reflected in distinct ICSS profiles.

431.1 ZINC ALTERS GLUTAMATE TOXICITY ON CORTICAL NEURONS. J. Koh and D.W. Choi. Department of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Chelatable zinc (Zn) may be co-released with glutamate in both normal synaptic transmission and in disease states such as hypoxia and epilepsy. We have recently shown that Zn blocks NMDA receptor-mediated excitation and toxicity on cultured cortical neurons, while often potentiating the excitation produced by quisqualate or AMPA. Elsewhere (S. Peters and D.W. Choi, this meeting), we described the effect of Zn on glutamate neuroexcitation. We report here the ability of Zn to alter glutamate neurotoxicity.

Exposure of mature cultured murine cortical neurons to 500 μM glutamate for 5 min invariably resulted by the following day in widespread neuronal disintegration and a substantial efflux of lactate dehydrogenase (LDH) to the bathing medium. Addition of 50 - 500 μM Zn to the glutamate exposure solution produced a concentration-dependent attenuation in both morphological neuronal damage and LDH efflux, with more than 50% reduction in neuronal injury at 300 - 500 μM. Higher concentrations of Zn were intrinsically neurotoxic, resulting in a "U-shaped" Zn concentration - neuronal injury relationship. Addition of 10 mM Mg did not reduce glutamate neurotoxicity.

As previously reported, 500 μM Zn completely blocked the neuronal damage by a 5 min exposure to 500 μM NMDA. However, this Zn concentration, which lacked intrinsic cytotoxicity, potentiated the minimal neuronal damage caused by low grade exposure to either quisqualate (30 μM for 5 min) or kainate (1 mM for 5 min), the former more strikingly than the latter.

Cortical neurons containing NADPH-diaphorase (an enzyme which co-localizes with somatostatin and NPY in forebrain) are selectively resistant to NMDA toxicity and, at the same time, selectively vulnerable to non-NMDA toxicity. Excess glutamate exposure destroyed this marker subpopulation in a non-selective fashion (i.e., in parallel with the general population). As expected, co-application of the NMDA antagonist APV with glutamate attenuated overall neuronal loss to a greater extent than diaphorase-containing neuronal loss. However, while co-application of 300 μM Zn with glutamate also reduced overall neuronal loss, it actually increased the destruction of the diaphorase-containing neuronal subpopulation.

These observations suggest that Zn, at concentrations which may be realized at excitatory synapses in vivo, could importantly modify the nature of glutamate neurotoxicity in disease states, reducing NMDA receptor-mediated toxicity, while perhaps increasing non-NMDA receptor-mediated toxicity.

Supported by NIH NS12151 and a Hartford fellowship to D.W.C.

431.2 ZINC MODULATES GLUTAMATE EXCITATION OF CORTICAL NEURONS. S. Peters and D.W. Choi. Department of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The transition metal zinc (Zn) is contained in neuronal synaptic vesicles in forebrain excitatory synaptic boutons, and is released in a calcium dependent manner by high K or electrical stimulation. In a recent study we showed that Zn attenuated NMDA receptor-mediated neuroexcitation, while usually potentiating the excitation produced by quisqualate and AMPA.

In the present study, we examined the effect of Zn on the neuroexcitatory action of glutamate, a broad spectrum agonist which is likely to be the actual substance co-released with Zn at many excitatory synapses. Intracellular recordings were made from murine cortical neurons in dissociated cell culture, using a defined recording medium containing 1 mM Mg.

Zn (500 μM to 1 mM), pressure ejected immediately prior to ejection of glutamate (100 μM), reversibly attenuated glutamate depolarizations to 57 +/- 11% (n=10) of control amplitude, while sometimes prolonging the response duration. In a direct comparison on several cells, Zn and the selective NMDA antagonist APV (500 μM) produced a similar degree of attenuation of glutamate responses. Zn blocked the initial rapidly desensitizing component that occurred in some glutamate responses, a phenomenon likely reflecting a predominant contribution from NMDA receptors. In the bath presence of the non-competitive NMDA antagonist ketamine (1 mM), glutamate responses were increased by prior ejection of Zn, consistent with the notion that the action of glutamate at quisqualate receptors can be potentiated by Zn.

To test the effect of Zn on spontaneous synaptic activity, cultures were bathed in Mg-free recording medium, which usually resulted in a high level of continuous and sometimes bursting excitatory activity. Pressure ejection of 1 mM Zn near the impaled cell soma attenuated spontaneous excitatory synaptic activity in about half of cells tested. In a direct comparison on several cells, Zn and APV (500 μM) usually produced parallel effects, both either reducing spontaneous excitatory activity or having no effect. On cells where Zn attenuated synaptic activity, pressure ejection of recording medium alone was without effect.

Our results support the hypothesis that Zn, co-released with glutamate at excitatory synapses, may modulate the postsynaptic action of glutamate, reducing its effect at NMDA receptors, and enhancing its effect at non-NMDA receptors.

Supported by NIH NS21628, NIH training grant NS07280 (S.P.), and a Hartford Foundation fellowship (D.W.C.).

431.3 GLUTAMATE TOXICITY IN A NEURONAL CELL LINE IS BLOCKED BY CALCIUM ION ANTAGONISTS, LITHIUM, OR MEMBRANE DEPOLARIZATION. T.H. Murphy*, R.L. Schnaar, J.T. Coyle, and A. Sastre, Depts. of Physiology, Pharmacology, and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore MD 21205.

Previous studies demonstrated that N18-RE-105 neuroblastoma hybrid cells bind [3H]-L-glutamate via a specific recognition site similar to the quisqualate preferring receptor of brain. Incubation of these cells in medium supplemented with quisqualate, L-glutamate (GLU), or ibotenate (agonists at the quisqualate receptor) resulted in greater than 70% cell lysis within 24 h, while incubation with kainate and N-methyl-D-L-aspartate (NMDLA) (agonists for other GLU receptors) had no effect. The cytotoxic effects of GLU were blocked by incubation in medium having a reduced free calcium ion concentration (<1μM). Consistent with a role for calcium in cytotoxicity, organic and inorganic voltage sensitive calcium channel (VSCC) blockers reduced GLU's toxicity.

L-glutamate induced depolarization. Intracellular recordings indicate that N18-RE-105 cell were depolarized (from control membrane potential of -47±1 mV) by "picospritzed" 50 mM GLU. Of 60 cells tested 45 were depolarized by GLU, with responses averaging 4.5±0.3 mV. Responses to other "picospritzed" GLU receptor agonists were evaluated only in GLU responsive cells. Nine/18 cells were depolarized (4.9±1.4 mV) by 5 mM quisqualate. Responses to 10 mM kainate were observed in 5/7 cells and averaged 3±0.6 mV. In contrast, "picospritzed" NMDLA (10 mM n=6) and L-aspartate (50 mM n=4) did not result in depolarization.

Desensitization. Repeated ("picospritzed") application of 50 mM GLU resulted in a 66±10% (n=8) reduction of the GLU response within 10-30 sec. Within 30-60 sec of cessation, the response to GLU returned to 107±8% (n=8) of control levels. Consistent with desensitization by repeated "picospritzing", *chronic* application of GLU (10 mM) did not produce any significant change in resting membrane potential over 0.5-1, 4-6, and 14-16 h of exposure; GLU-induced cell lysis was maximal during 12-18 hours of exposure.

Chronic depolarization blocks L-glutamate's toxicity. When N18-RE-105 cells were exposed to GLU in the presence of depolarizing agents (110 mM KCl, 250 μM ouabain, or 50 μM veratridine), GLU-induced toxicity was reduced 49-89%, (cell death in depolarized cultures in the absence of GLU did not increase more than 15% above control levels). Membrane potentials measured in the presence of the protective concentrations of depolarizing agents (and the absence of GLU) were between -8 and -18 mV, while untreated cells averaged -46±2 mV. Blockade of GLU toxicity by chronic depolarization may be consistent with a role for VSCC's since some types can be inactivated at depolarized potentials (Nowycky, M.C., et al., *Nature*, 316:440(1985).

Lithium blocks toxicity. Lithium (20 mM) blocked greater than 70% of GLU toxicity, without having an effect on control cell viability or membrane potential, suggesting a role for inositol phosphates in GLU toxicity. The absence of a direct correlation between GLU-induced depolarization and GLU toxicity in the N18-RE-105 cell line suggests that second messengers other than membrane potential may be involved. *Supported by Grants NS 13584 and GM 0726.*

431.4 TETRAHYDROAMINOACRIDINE (THA) AT HIGH CONCENTRATION ANTAGONIZES N-METHYL-D-ASPARTATE NEUROTOXICITY AND NEUROEXCITATION. C.J. Davenport, J. Koh, S. Peters and D.W. Choi. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Recently A.B. Young and colleagues (reported this meeting) found that THA can displace 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP) binding to rat cortical membranes. Based on their finding, and in view of several studies suggesting a link between the PCP receptor site and the N-methyl-D-aspartate (NMDA) receptor complex, we decided to see if THA could antagonize the toxic or excitatory actions of NMDA on cortical neurons.

A 5 min exposure of mature (15-21 d in vitro) dissociated murine neocortical cell cultures either to 500 μM NMDA or 500 μM quisqualate, resulted 1 d later in widespread neuronal disintegration and a large efflux of lactate dehydrogenase (LDH) into the bathing media. Addition of 1 - 3 mM concentrations of THA to the NMDA exposure solution almost completely blocked both the morphological evidence of neuronal cell loss and its biochemical correlate, LDH efflux, but did not much reduce quisqualate-induced neuronal loss. Lower (100 - 300 μM) concentrations of THA afforded only slight protective effect against NMDA. THA bears structural resemblance to the potassium channel antagonist 4-aminopyridine (4-AP), but addition of 3 mM 4-AP to the exposure solution provided no protection against NMDA-induced neuronal injury. 3 mM physostigmine showed some weak and variable neuron-protective efficacy against NMDA.

Intracellular electrophysiological experiments supported an action of THA on NMDA receptors. Pressure ejection of THA (500 μM to 2 mM) usually produced an increase in membrane resistance and a transient membrane depolarization of several mV, but also substantially attenuated the subsequent depolarizing response to pressure ejection of NMDA. Physostigmine (2 mM) was less effective in attenuating the NMDA response, and 4-AP produced little or no block.

The potency of THA as an antagonist of NMDA receptor-mediated toxicity and excitation appears low, but the possibility exists that this novel action might, under some circumstances, be added to the predominant anticholinesterase action of the drug. In any case, study of the structure-activity implications of this action may yield insights useful in the development of other more potent NMDA antagonists.

Supported by NIH NS12151, NIH training grant NS07280 (S.P.), and a Hartford Foundation fellowship (D.W.C.).

431.5 1,2,3,4-TETRAHYDRO-9-AMINOACRIDINE AND OTHER ACRIDINE DERIVATIVES ARE DISPLACERS OF SPECIFICALLY BOUND [^{3}H]-N-(1-[2-THIENYL]CYCLOHEXYL)-3,4-PIPERIDINE. A.B. Young, R.L. Albin*, M.S. Albin*,†, J.B. Penney, J.T. Greenamyre, W.F. Maragos. Dept. of Neurology*, University of Michigan, Ann Arbor MI 48104, and †Dept. of Anesthesiology, UTHSCSA, San Antonio, TX 78284.

1,2,3,4-tetrahydro-9-aminoacridine (THA) has recently been reported to substantially ameliorate the cognitive deficits of Alzheimer's disease (AD; Summers et al., NEJM 315:1241, 1986). THA has centrally and peripherally acting cholinesterase-inhibitory activity. Its beneficial effect has been attributed to this mechanism even though prior trials of other cholinesterase-inhibitors produced little or no benefit in AD. THA, however, has a complex pharmacology. It reduces the duration of anesthesia caused by the dissociative anesthetics, phencyclidine (PCP) and ketamine (Kt), and the emergence responses associated with Kt.

PCP receptor density is decreased in AD brains. The PCP receptor is closely linked to the NMDA receptor, which is particularly dense in the hippocampus where AD pathology is severe, and may be involved in synaptic mechanisms underlying learning and memory. It has been hypothesized that endogenous excitotoxins acting at the NMDA receptor may be involved in the pathogenesis of AD.

Choi and colleagues have shown (this meeting) that THA blocked NMDA induced cytotoxicity in cultured murine neurons, and that physostigmine was a weaker inhibitor of NMDA induced cytotoxicity.

We measured the ability of THA and several related compounds to displace specifically bound [3H]-N-(1-[2-thienyl]cyclohexyl)-3,4-piperidine (TCP), a potent PCP analogue, from rat brain membranes. The IC_{50}'s were: THA, 30 μM; 9-aminoacridine, 10 μM; proflavin, 30 μM ; physotigmine and edrophonium ~ 200 μM; neostigmine, 4-aminopyridine, 3,4-diaminopyridine and 3-aminopyridine, >> 100 μM. 300 μM THA did not displace [3H]glutamate from NMDA receptors as determined by quantitative autoradiography.

Our results demonstrate that THA and other acridines interact with the dissociative anesthetic receptor albeit, somewhat weakly. Other cholinesterase inhibitors were considerably weaker as TCP displacers. These results, together with those of Choi and colleagues, suggest that THA may exert some of its effects by interacting with the PCP/NMDA receptor complex. The beneficial effects of THA in AD may be attributable to its modulation of both cholinergic and glutamatergic neurotransmission.

Supported by USPHS grants AG 06155 and NS 07222.

431.6 HYPOXIA-ISCHEMIA CAUSES GLUTAMATE-LIKE NEUROPATHOLOGICAL CHANGES IN INFANT RAT BRAIN. J.M. Olney*, G.E. Gray*, G. Frierdich*, K. Shahid Salles, J. Labruyere*, M.T. Price, and J.W. Olney (SPON: J. Martin). Dept. of Psychiatry, Washington University Medical School, St. Louis, MO 63110.

The excitatory transmitters, glutamate (Glu) and aspartate (Asp), are neurotoxins capable of destroying CNS neurons by an excitotoxic process. The acute cytopathological changes in the immature retina or hypothalamus induced by exogenous Glu develop very rapidly, leaving central neurons in an advanced irreversible stage of necrosis only 2-4 hrs following subcutaneous Glu administration. This acute fulminating excitotoxic process occurs by a Na^+ and Cl^- dependent but Ca^{++} independent mechanism. A more delayed excitotoxic process in which Ca^{++} plays a role has also been described.

It is thought that an excitotoxic mechanism may underlie brain damage associated with anoxia-ischemia: Glu antagonists reportedly can prevent such damage, and it has been shown that endogenous Glu & Asp accumulate extracellularly in rat brain during experimental cerebral ischemia. Efforts to prevent such degeneration by in vivo administration of Glu antagonists have, thus far, concentrated primarily on models in which the degenerative process affects a single type of neuron--the CA-1 hippocampal pyramid--and the degenerative process is of the delayed type, occurring over a 4 day period. Although severe anoxia-ischemia can certainly cause more acute brain damage, prior studies have not established that such damage has pathognomonic characteristics of acute Glu-like excitotoxic damage.

Here we demonstrate in the infant rat that unilateral occlusion of the carotid artery followed by exposure to hypoxic or hypobaric conditions causes neurons in several brain regions to degenerate by an acute fulminating process that appears identical by light or electron microscopy to that seen in the infant retina or hypothalamus following subcutaneous administration of exogenous Glu. This may be a useful model for clarifying whether the ability of Glu antagonists to protect against anoxic-ischemic neuronal degeneration in vivo is limited to the delayed type of degeneration affecting CA-1 hippocampal neurons, or extends also to an acute fulminating type of degeneration to which other neurons are susceptible. Moreover, it is an excellent model for studying pathogenic mechanisms of, and prophylactic approaches to, a serious childhood neurodegenerative disorder, cerebral palsy. Supported by Research Scientist Award MH38894 (JWO) and a grant from Pfizer Pharmaceutical.

431.7 PREVENTION OF ACUTE EXCITOTOXICITY IN EMBRYONIC CHICK RETINA BY AN INHIBITOR OF Cl^- TRANSPORT. W.J. Nicklas, G.D. Zeevalk, V.P. Muzithras* and A.G. Hyndman. UMDNJ-Robert Wood Johnson Medical School and Rutgers University, Piscataway, NJ 08854.

Previous studies have shown that kainate (KA) and other excitotoxins incubated with susceptible brain preparations can cause multiple neurochemical sequelae, such as decreased glutamine (GLN) synthesis and release of certain amino acids, e.g., glutamate (GLU); these in vitro effects were thought to be associated with swelling of cellular elements and could be prevented by treatment with the Cl^-/HCO_3^- antiport inhibitor, DIDS. In the present study, central posterior retina of embryonic (E13) chick was incubated in a Krebs-Ringer-bicarbonate medium with various concentrations of KA (2-200 μM) in the presence and absence of DIDS (300 or 600 μM). Amino acid (AA) levels in the tissue and incubation medium were measured and correlated with histological responses. At 2 μM changes in AA were minimal; at 25 μM there was a measurable increase in medium GLN and taurine (TAU). At 50 μM KA, GLU, GLN, TAU, ALA and GABA were significantly increased in the medium. These trends were further increased at 100 and 200 μM KA. Coincubation with 300 μM DIDS substantially, but only partially, prevented the release induced by 50 μM KA; 600 μM DIDS offered complete protection. Histological damage was found at all concentrations of KA tested; however, just as with the AA findings there was a gradation of response seen both radially and longitudinally. The radial damage was typical of KA-induced retinal lesions, i.e., mainly somal swelling in the mid and inner regions of the inner nuclear layer (INL) and vacuolization in the inner plexiform layer (IPL). Increasing concentrations of KA produced more prominent somal swelling in the INL, an increase in IPL vacuolization and involvement of other retinal populations (displaced amacrines and somas in upper regions of the INL). At 200 μM KA ganglion cell swelling was noted. There was a sharply defined regional sensitivity to KA along the length of central posterior retina. 2 μM KA produced a typical lesion along about 50% of the tissue length. Increasing KA correspondingly increased the length of retina involved; at 200 μM KA the entire length of retina was maximally affected. A graded response was also seen in areas less sensitive to KA. Consistent with the biochemical findings, 300 μM DIDS partially, and 600 μM DIDS completely, blocked the lesion caused by 50 μM KA. About 80% of the central posterior retina was maximally lesioned by 50 μM KA with 20% showing mild to moderate effects. Earlier studies by others showed that deletion of Cl^- from the incubation offered similar protection. The present experiments indicate that Cl^- may participate in the acute excitotoxic response via mechanisms more complicated than simple passive diffusion.

431.8 PHARMACOLOGICAL PROPERTIES OF A KAINIC ACID-PREFERRING RECEPTOR ON OFF-BIPOLAR CELLS IN THE CHICKEN RETINA. I.G. Morgan (SPON: R.F. Mark). Centre for Visual Sciences and Research School of Biological Sciences, Australian National University, GPO Box 475, Canberra City, ACT 2601, Australia.

The pharmacological properties of a kainic acid-preferring receptor on bipolar cells in the chicken retina were examined by following the cell swelling and necrosis induced by intravitreal injections of excitatory amino acid agonists and antagonists. Acute exposure to low concentrations of kainic acid (5-10 um) caused selective swelling of bipolar cells in the chicken retina. Long term exposure resulted in destruction of the cells, with elimination of OFF-responses at the ganglion cell level, suggesting that they are OFF-bipolar cells (Dvorak, D.R. and Morgan, I.G. Neuroscience Letters 36:249, 1983). In contrast, 1000-fold higher doses of N-methyl-D-aspartic acid, quisqualic acid and RS-alpha-amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid had no effect on these cells, although they were effective excitotoxins in the inner retina, where RS-alpha-amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid was equipotent with kainic acid. While the lack of effect of quisqualic acid could be explained by active uptake, there is no evidence that N-methyl-D-aspartic acid and RS-alpha-amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid are already taken up by neural cells. Bromowillardine, which is a powerful agonist at the kainic acid-preferring receptors on immature rat dorsal root primary afferent C-fibres (Agrawal, S.G., and Evans, R.H. Brit. J. Pharmacol. 87: 345, 1986), had no obvious effect on the bipolar cells, at doses up to 100 times those of kainic acid, but exerted substantial toxicity in the inner retina. Only at doses 1000 times those of kainic acid did it affect the bipolar cells. Willardine was without effect on the bipolar cells. The effects of kainic acid on the bipolar cells were blocked effectively by piperidine-2, 3-dicarboxylic acid, but gamma-D-glutamylglycine and gamma-aminomethanesulphonic acid were also effective, albeit not potent antagonists. D-O-Phosphoserine was a weak antagonist of the effects of kainic acid on the bipolar cells. 2-Amino-5-phosphonopentanoic acid and glutamic acid diethylester were without effect. The selective effectiveness of kainic acid and piperidine-2,3-dicarboxylic acid at the receptors on the OFF-bipolar cells provides an interesting model for studying excitatory amino acid transmission and excitotoxicity.

431.9 NADPH DIAPHORASE HISTOCHEMISTRY IN RABBIT RETINA: EFFECTS OF KAINIC ACID. S.M. Sagar. Neurology Service, V. A. Medical Center, and University of California, San Francisco, CA 94143.

We have previously shown that NADPH diaphorase (NADPHd) histochemistry in the rabbit retina selectively stains two classes of amacrine cells which can be distinguished by cell body size and intensity of staining. In the brain, NADPHd-reactive neurons have been shown to be resistant to a variety of metabolic insults, including the N-methyl-D-aspartate (NMDA) class of excitatory neurotoxins. As an initial step in the evaluation of the effects of neurotoxins on retinal NADPHd neurons, kainic acid (KA) was administered by intravitreal injection to one eye of adult, male rabbits; the opposite eye received sterile saline as a control. Ten days later, the retinas were removed and hemisected vertically. Half of each retina was fixed in 4% paraformaldehyde and processed for NADPHd histochemistry; the other half was assayed for dopamine (DA) by HPLC with electrochemical detection and for protein.

At doses of 30 and 60 nmol KA, the DA concentration was reduced by 80% or more; and the large, darkly staining NADPHd-reactive cell type was substantially destroyed. However, the smaller cells were relatively spared. Because the large cells and their processes were removed, the morphology of the small cells was clarified. These cells have their dendritic arbor in the outermost sublamina of the inner plexiform layer (IPL), implying that the large cells give rise to all of the processes in the middle sublamina of control retinas. Occasional large NADPHd-reactive cells survived. A notable feature of these cells revealed in KA injected retinas is that they have straight, sparsely branching varicose processes which extend for several mm in the IPL. At a dose of 120 nmol, many of the small cells are destroyed also.

Therefore, the small NADPHd reactive neurons are relatively resistant to the actions of KA, and the toxin is a useful morphologic tool to expose anatomic features of these cells. The larger cells are about equally sensitive to KA as dopaminergic cells, so that resistance to excitatory neurotoxins is not a universal feature of NADPHd reactive neurons. The two NADPHd reactive cell types may receive different excitatory amino acid inputs or express different classes of receptors. Future studies employing excitatory neurotoxins other than KA will explore this hypothesis further.

EXCITATORY AMINO ACIDS: PHENCYCLIDINE INTERACTION

432.1 INTERACTION OF L-GLUTAMATE (L-GLU) AND MG^{2+} WITH PHENCYCLIDINE (PCP) RECOGNITION SITES IN RAT BRAIN PROVIDES EVIDENCE FOR MULTIPLE AFFINITY STATES OF THE PCP/ N-METHYL-D-ASPARTATE RECEPTOR COMPLEX. Matthew A. Sills, Patricia A. Loo*, Albert F. Braunwalder* and Michael Williams. Drug Discovery Division, Research Department, CIBA-GEIGY Pharmaceuticals, Summit, NJ 07901.

Recent evidence suggests that a PCP/NMDA receptor complex exists in rat forebrain, which is reminiscent of that demonstrated for GABA and the benzodiazepines. In well washed membranes, L-GLU produces significant increases (400%) in the binding of [^{3}H]TCP in the rat forebrain (Loo et al. Eur. J. Pharmacol. 123: 467). Further studies have also indicated an involvement of the divalent cation, Mg^{2+}. To further investigate these effects, a series of saturation experiments were conducted using approximately 24 concentrations of [^{3}H]TCP over the range 0.1-130 nM under four conditions: the absence of L-GLU and Mg^{2+} (basal), the presence of Mg^{2+} (30 μM), the presence of L-GLU (100 μM) and the presence of both Mg^{2+} and L-GLU. A single component of binding was revealed under basal conditions with a Kd value of 112 nM and an apparent Bmax of 2705 fmole/mg protein. In the presence of 30 μM-Mg^{2+} or 100 μM-L-GLU, a single component of binding was again observed with an apparent Bmax similar to that seen under basal conditions. However the Kd value was reduced to 53 nM by Mg^{2+} and to 20 nM by L-GLU. Inclusion of L-GLU in the presence of Mg^{2+} however, resulted in the appearance of two binding components, one of high affinity (Kd = 4 nM) and a lower affinity site (Kd = 92 nM), the latter similar in affinity to the site measured under basal conditions. The respective apparent Bmax values for these sites were 1095 and 1708 fmole/mg protein. The sum of these (2805 fmole/mg protein) is in good agreement with the number of sites seen under the other assay conditions. Examination of inhibition curves produced by PCP-like compounds shows that two binding components are present whose percentages and IC-50 values vary depending on the presence of L-GLU or Mg^{2+} (Braunwalder et al. Fed. Proc. 46: 1310). These results are consistent with the concept that L-GLU and Mg^{2+} are stabilizing the formation of a high affinity state of the PCP recognition site in rat forebrain tissue.

432.2 MODULATION OF PHENCYCLIDINE RECEPTORS BY N-METYL-D-ASPARTATE RECEPTOR LIGANDS. D.C. Javitt, R. Sircar and S.R. Zukin, Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461.

Numerous biochemical and behavioral studies have suggested that phencyclidine (PCP) and related dissociative anesthetics exert their neurobehavioral effects in rats by interacting with a specific PCP/σ receptor. Electrophysiological and biochemical studies have shown that PCP, while not an N-methyl-D-aspartate (NMDA) receptor ligand, non-competitively inhibits NMDA receptor mediated potentials. Anatomical studies have shown colocalization of PCP and NMDA receptors in the limbic system. In order to investigate further the interaction between PCP and NMDA receptors, we undertook a series of binding studies using D(-)2-amino-5-phosphonovaleric acid (D(-)AP5) and [^{3}H]TCP as probes of NMDA and PCP receptors, respectively. D(-)AP5, a potent and selective direct NMDA receptor anatagonist, inhibited [^{3}H]TCP binding in a dose dependent fashion by selectively decreasing the apparent number of [^{3}H]TCP binding sites without altering their affinity for [^{3}H]TCP. Extensive washing of the rat brain membranes followed by freezing and thawing also decreased the apparent number of PCP receptors without altering their affinity. In both D(-)AP5 treated and frozen-thawed membranes, glutamate receptor agonists were able to partially restore binding with the order of potency L-glutamate > NMDA > quinolinate > quisqualate. Kainate was ineffective at restoring binding of [^{3}H]TCP. In thrice-washed rat forebrain homogenate, D(-)AP5 displaced [^{3}H]TCP with an IC50 of 21 μM. Further washing and freezing and thawing of the membranes decreased the IC50 to 1 μM. NMDA receptor agonists increased the IC_{50} of D(-)AP5 vs. [^{3}H]TCP. Schild analyses suggested K_Ds of 0.11, 0.63, and 11.0 μM for L-glutamate, NMDA, and quisqualate, respectively. These values are in good agreement with the reported affinity of these ligands for the NMDA receptor. The ability of direct NMDA receptor antagonists to decrease the apparent number of PCP receptors and of NMDA receptor agonists to increase the apparent number of receptors suggests that PCP receptors may be solely localized to a site on the active conformation of the NMDA receptor complex. In light of the ability of PCP and related compounds to non-competitively inhibit the electrophysiological effects of NMDA, this site may overlap or reside within the open channel of the NMDA related ionophore. Such an interaction would be analogous to the interaction between picrotoxin and $GABA_A$ receptors. A recently described endogenous brain peptide with PCP-like receptor-binding and anti-NMDA biological activity (S.R. Zukin et al., Brain Research, in press) may regulate NMDA receptor mediated processes in vivo.

432.3 D-APV AND PHENCYCLIDINE DECREASE CA1 PYRAMIDAL CELL EXCITATION PRODUCED BY NORMORPHINE IN THE HIPPOCAMPAL SLICE. E.S. Swearengen, A.T. Malouf and C. Chavkin. Department of Pharmacology, University of Washington, Seattle, WA 98195.

Electrophysiological studies suggest that opioids increase hippocampal pyramidal cell excitation by reducing inhibitory neurotransmitter release from GABA-ergic interneurons. Opioid-induced pyramidal cell disinhibition also involves NMDA receptor activation as demonstrated by the ability of D-2-amino-5-phosphonovalerate (D-APV), a selective NMDA receptor antagonist, to partially inhibit the increase in CA1 pyramidal cell excitability produced by normorphine (Swearengen and Chavkin, Neurosci. Lett., in press). Lodge and coworkers have demonstrated that PCP non-competitively inhibits NMDA receptor activation in cat and rat spinal cord. Effects of PCP on hippocampal CA1 pyramidal cells have also been studied (Malouf, et al., this volume). We have therefore compared the ability of D-APV and PCP to reduce CA1 pyramidal cell excitation produced by normorphine (NOR).

Extracellular recordings were made from rat hippocampal slices (500μm); CA1 population spikes were evoked by Schaffer collateral stimulation. The stimulus intensity required to produce 1/2 maximum primary response (S1/2) and afterpotential amplitude were measured. NOR (10μM) added to the superfusion buffer decreased the S1/2 18.2 ± 2.6% (mean ± SEM, n = 9) and produced a 3.18 ± 0.26 mV afterpotential (mean ± SEM, n = 11). D-APV partially inhibited NOR-induced afterpotentials (IC_{50} = 4.8μM) with maximal inhibition of 65.8 ± 2.6% (mean ± SEM, n = 4) seen at 100μM. D-APV did not significantly block the opioid effect on primary response S1/2 primary response S1/2. In contrast to D-APV, PCP reversed the NOR-induced decrease in S1/2 to 108.0% of control, with an IC_{50} of 6.6μM. PCP (100-300μM) also completely inhibited the NOR-induced afterpotential with an IC_{50} of 15.1μM. We conclude that the NOR-induced afterpotential has an NMDA-mediated component and an additional excitatory component both of which are sensitive to PCP. This suggests that the actions of PCP, at this concentration, are not limited to NMDA receptor inhibition. Interestingly, NOR-induced afterpotentials in the dentate granule cell region were completely blocked by 50mM D-APV, suggesting that there exist intrinsic differences in the pharmacology and/or circuitry of these two regions.

The ability of PCP to completely inhibit afterpotentials and to decrease tissue sensitivity to stimulation is thought to be due to anesthetic effects on sodium channels. Intracellular CA1 pyramidal cell recordings reveal that PCP (1mM) abolished synaptically driven spikes, reduced the amplitude and increased spike width following +0.5nA, 100ms current injection. The same concentration of PCP also abolished evoked field potentials recorded extracellularly in the absence of NOR. These effects were completely reversible.

3-PPP, in intracellular recordings, had no effect on NMDA-induced depolarizations at 100mM, a concentration below the threshold for its anesthetic actions. In extracellular recordings, 3-PPP (100μM) partially blocked the NOR-induced afterpotential. The mechanism of 3-PPP action in this system is not clear but is unlikely to be NMDA receptor inhibition.

This study was supported by NIH Grant NS-23483, a PMA research starter grant, and PHS NRSA 5-T32 GM0720 from NIGM.

432.4 ELECTROPHYSIOLOGICAL ANALYSIS OF HIGH AFFINITY (+)SKF-10047/SIGMA AND PCP/SIGMA LIGANDS ON CA1 PYRAMIDAL NEURONS IN VITRO. A.T. Malouf, E.S. Swearengen and C. Chavkin, Department of Pharmacology, University of Washington, Seattle, WA 9819

[3H](+)SKF-10047 has been shown to bind to high and low affinity sites in the hippocampus and other brain regions. The psychotomimetic properties of sigma ligands and dissociative anesthetics such as PCP are mediated through the low affinity site. Lodge and coworkers have shown that ligands acting at this site inhibit NMDA responses. These compounds have also been reported to have effects on potassium and possibly other ion conductances. As yet, no physiological or behavioral effects have been reported to be mediated by high affinity sites in the CNS. We have compared the physiological and pharmacological actions of the high affinity sigma ligands, (+)3-PPP (3-[3-hydroxyphenyl]-N-(1-propyl)piperidine), DTG (ditolylguanidine) and (+)SKF-10047 to PCP in area CA1 of the rat hippocampal slice

Bath application of (+)3-PPP or (+)SKF-10047 at concentrations below 100 μM produce no change in spontaneous activity, membrane potential, input resistance, spike amplitude or width, AHP following a +1 nA/200 ms current pulse, threshold for orthodromicaly driven spikes or the amplitude or duration of the resulting IPSP. 100 μM DTG and PCP or 1 mM (+)3-PPP produced a decrease in spontaneous activity, an increase in spike width, fewer spikes following depolarizing current injection and an increase in threshold for orthodromically driven spikes. 1 mM DTG or PCP dramatically reduced spike amplitude and the number of spikes per current pulse and abolished the synaptically driven spike and the associated EPSP/IPSP.

Extracellular recordings of CA1 field potentials also demonstrated that bath application of PCP, (+)SKF-10047, DTG and (+)3-PPP at concentrations less than 100 μM had little or no effect on either sensitivity to stimulation of Schaffer collaterals or the amplitude of the orthodromically evoked primary population spike. Higher concentrations (1 mM) of these compounds produced a complete inhibition of the primary spike which fully recovered within 60 min following washout.

The ability of (+)3-PPP, DTG and PCP to inhibit NMDA induced activation was also tested. NMDA was applied to the pyramidal cell dendrites in stratum radiatum via pressure pipette filled with 100 μM drug solution. Bath applied (+)3-PPP (1 mM) or DTG (100 μM) produced no change in the amplitude or duration of the NMDA induced depolarization, while PCP (10 μM) completely inhibited this response on the same cell. The NMDA response recovered slowly following washout of PCP.

Except for the PCP inhibition of NMDA responses, these compounds appear inactive on CA1 pyramidal neurons at concentrations less than 100 μM. Concentrations greater than or equal to 100 mM alter ion conductances, and probably represents the anesthetic properties of these compounds. The lack of effect of DTG and 3-PPP on the NMDA receptor suggests that further investigation will be required to elucidate the function of high affinity (+)SKF-10047 sites in the hippocampus.

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432.5 **NMDA AND KAINATE INTERACTION AT THE EXCITATORY AMINO ACID RECEPTORS IN ISOLATED RAT RETINAL GANGLION CELLS: EFFECTS OF PHENCYCLIDINE AND MK801**. A. Karschin*, E. Aizenman & S.A. Lipton (SPON: C. Applegate). Max-Planck-Institut für Hirnforschung, 6000 Frankfurt 71, F.R.G. and Div. of Neuroscience, The Children's Hospital, and Dept of Neurology, Harvard Medical School, Boston, MA 02115.

We have previously shown that glutamate and its analogues, kainate, quisqualate, and NMDA, produce responses of varying amplitudes in isolated rat retinal ganglion cells (Aizenman et al., Soc. Neurosci. Abstr. 12:56; 1986). In order to further investigate the pharmacological properties of the postulated amino acid receptor channel complexes we studied the interactions between NMDA and kainate in solitary ganglion cells from the rat retina by means of the whole-cell patch-clamp technique. Ganglion cells were retrogradely labelled *in situ* by injection of the fluorescent marker granular blue into their projection sites. The application of drugs during recording was performed by microperfusion from glass pipettes.

In the absence of extracellular Mg^{++}, NMDA (200 μM, the maximal non-desensitizing dose) generated inward currents (-10 to -40 pA) in 68% of the cells under study when voltage-clamped at their resting potential of -60 mV. In contrast, kainate (125 μM) produced larger non-desensitizing currents (-80 to -750 pA) in all cells tested (V_H=-60 mV). When applied together, NMDA appeared to antagonize the kainate responses by approximately 50%, even in cells having no measurable response to NMDA alone.

The presumptive NMDA channel blockers PCP (phencyclidine-d5-hydrochloride; 100 μM) and MK801 (10 μM) reversibly antagonized responses evoked by NMDA (200 μM). In contrast neither PCP nor MK801 influenced the responses evoked by kainate at the given concentrations. Furthermore, in the presence of NMDA and PCP, kainate generated large inward currents which were comparable in amplitude to those generated by the addition of kainate and NMDA together. Similar results were found when MK801 was substituted for PCP. Thus, kainate continued to elicit currents at a time when NMDA-activated conductances were blocked by either PCP or MK801.

Based on these findings, this system will be useful in studying the ionophores associated with excitatory amino acid receptors.

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432.6 A COMPARISON OF THE ANTICONVULSANT EFFECTS OF PHENCYCLIDINE AND ANTAGONISTS OF THE N-METHYL-D-ASPARTATE RECEPTOR IN THE MOUSE. M.E. Nevins and S.M. Arnolde*. CNS Diseases Research, G.D. Searle & Co., Skokie, IL 60077.

Phencyclidine (PCP) receptor agonists and N-methyl-D-aspartate (NMDA) receptor antagonists have several properties in common. These include anticonvulsant, anxiolytic and neuroprotective activity as well as stereotypy and memory impairment. It has been proposed that the PCP and NMDA receptors are linked to a common receptor/ion channel complex, and that some of the pharmacological effects of PCP are mediated through PCP receptor regulation of NMDA actions. If so, then PCP should have a profile of anticonvulsant activity similar to that of the NMDA antagonists, 2-amino-7-phosphonoheptanoic acid (AP7) and 3-[(+)-2-carboxypiperazin-4-yl]-propyl-1-phosphonate (CPP). Tonic hindlimb extension (THLE) was produced in male CD-1 mice by four methods; pentylenetetrazol (PTZ) administration (110 mg/kg s.c.), 3-mercaptopropionic acid (3-MPA) administration (48 mg/kg i.p.), maximal (50 mA) electoconvulsive shock (ECS) and threshold (12 mA) ECS. CPP and AP7 were administered s.c. 45 min prior to, and PCP was administered i.p. 30 min prior to administration of the convulsant. Groups of 10 mice per dose were used to calculate ED50's for each drug in each of the convulsant models. The results were as follows:

	ED50 (mg/kg)		
Convulsant	PCP	CPP	AP7
PTZ	3.8	0.9	17.4
3-MPA	2.0	1.5	13.2
12mA ECS	2.2	1.8	23.0
50mA ECS	5.3	6.1	82.6

PCP, CPP and AP7 each produced a dose-dependent inhibition of THLE produced by the four different convulsant stimuli. In the PTZ and 3-MPA models, the slopes of the three drugs did not differ. However, in both of the ECS models, the slope of PCP was different from that of CPP and AP7. In addition, the three drugs were similar in that they each antagonized 50mA ECS less potently than they antagonized the other three convulsant stimuli. For each drug, significantly higher doses were required to antagonize 50mA ECS than any of the other convulsant stimuli. The finding that the anticonvulsant profile of PCP is similar to that of the NMDA antagonists CPP and AP7 supports the idea that the anticonvulsant effects of PCP may be exerted through a mechanism of action common with that of the NMDA antagonists.

432.7 MK-801 POWERFULLY PROTECTS AGAINST N-METHYL ASPARTATE NEUROTOXICITY. M.T. Price, J.W. Olney, K. Shadid Salles, J. Labruyere and G. Frierdich*. Washington University School of Medicine, St. Louis, MO. 63110.

The excitatory amino acids (EAA), glutamate (Glu) and aspartate and related analogs--known collectively as excitotoxins--have neurotoxic activity mediated through excitatory synaptic receptors. Several EAA receptor subtypes have been identified and named according to the agonist (N-methyl-asparate, kainate or quisqualate) with which they preferentially interact. A number of EAA antagonists have been identified, the most potent and specific being those that block the excitatory and neurotoxic activity of N-methyl-aspartate (NMA). EAA antagonists have been classified as follows: 1) mixed EAA antagonists, 2) competitive NMA antagonists, and 3) non-competitive NMA antagonists. Phencyclidine (PCP), a non-competitive NMA antagonist (Anis et al., Br J Pharm 79, 565, 1983), is the most powerful antagonist of EAA neurotoxicity previously described (Olney et al., Neurosci Lett, 68, 29, 1986). Here we report that MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo (a,d) cyclohepten-5,10-imine], which resembles PCP in electrophysiological and receptor binding properties (Wong et al., PNAS, 83, 7104, 1986), is 5 times more powerful than PCP in preventing NMA neurotoxicity in the chick embryo retina, that its antagonism is specific for NMA toxicity, is non-competitive and does not entail inhibition of EAA receptor binding.

Endogenous excitotoxins have been implicated in several neuropathological conditions, including brain damage associated with epilepsy, anoxia-ischemia and hypoglycemia. While more than one EAA receptor subtype may be involved in the pathophysiology of these conditions, the NMA receptor may be of particular importance as it is the most abundant EAA receptor subtype in mammalian brain. Given the extreme potency of MK-801 in preventing NMA receptor-mediated neurotoxicity and recent evidence for its ability to prevent either seizure-mediated (Clifford et al., this meeting) or anoxic-ischemic (Foster et al., Brit J Pharm 90, 9P, 1987; Fuller et. al., this meeting) neuronal degeneration in vivo, it may be a valuable neuroprotective agent in clinical neurology. Supported by RSA MH 38894 (JWO), ES 07066, DAMD 17-86-C-6010 and a grant from the Washington Univ/Monsanto Biomed Res Fund. The MK-801 was generously supplied by Merck Sharp and Dohme Res. Labs.

432.8 COMPARATIVE EFFECTS OF PHENCYCLIDINE, KETAMINE AND MK-801 ON THE RAT ELECTROENCEPHALOGRAM (EEG). J. French, P. Ho* and E.F. Domino. Department of Pharmacology, University of Michigan, Ann Arbor, MI 48109-0010.

Characteristic EEG effects from the neocortex and hippocampus were compared following phencyclidine (PCP), ketamine and MK-801. A total of 6 adult, male, Sprague-Dawley rats were chronically implanted with bipolar EEG and temporalis muscle electrodes. Each rat was placed in a plexiglass chamber 30 cm in diameter by 29 cm deep and recordings were obtained on polygraph paper and FM magnetic tape. Additionally, gross body movements and EEG data were collected simultaneously on a video/analog cassette recorder. Cumulative doses of PCP (3.2, 10, 32 and 56 mg/kg), ketamine (10, 32, 100 and 180 mg/kg) and MK-801 (1, 3.2, 10 and 32 mg/kg) as base content, were administered i.p. every 15 min. Rats were used every 2-4 days to allow for drug elimination. A Latin Square design was used such that the order of drug presentation was random for each rat. Maximal doses were based on 75-80% of the estimated LD_{50} for each compound.

Many behavioral and EEG features common to all three compounds were observed. Small doses produced side to side head movements and stereotyped circling behavior which were accompanied on the EEG by large amplitude irregular activity from the hippocampus and large slow waves (1-3 Hz) from the neocortex. The amplitude of the electromyogram (EMG) also was increased. After intermediate doses of these agents, most rats were unable to support themselves. The amplitude and incidence of large, irregular activity in the hippocampus and slow waves in the cortex increased. After even larger doses, episodic sharp waves began to appear from both EEG leads and the animals were typically unable to right themselves. For PCP, this EEG sharp wave activity was correlated with the EMG. The largest dose of each compound produced an increase in the frequency and occurrence of all aberrant wave forms in both leads. However, PCP produced pronounced but brief EEG and EMG seizure activities and ketamine produced effective, general anesthesia. After 1 hr post dosing, sharp activity decreased while the incidence of background 15-20 Hz activity increased in both leads. After 7 hr gross behavior and EEG partially recovered and by 24 hr, returned to baseline levels.

It is concluded that while all three agents have similar EEG and gross behavioral features, depending on dose, there are distinct differences which make a simple classification difficult. (Supported in part by NIDA grant DA 1531.)

432.9 THE PHENCYCLIDINE (PCP) RECEPTOR ACYLATING AGENT, METAPHIT, POTENTIATES 2-AMINO-7 PHOSPHONOHEPTANOATE (AP7) INDUCED STEREOTYPED BEHAVIOR AND ATAXIA IN RATS. R.P. Compton[1]* W.F. Hood[1], P.C. Contreras[1], T.L. O'Donohue[1], A.E. Jacobson[2]*, M.F. Rafferty[2]*, K.C. Rice[2]*, R.A. Lessor[2]* and J.B. Monahan[1] [1]Searle Research & Development, Chesterfield, Mo. 63198; [2]Laboratory of Chemistry, NIADDK, Bethesda, MD. 20205.

The competitive N-methyl-D-aspartate (NMDA) antagonist AP7 has been shown to induce phencyclidine-like stereotyped behavior and ataxia in rats in a dose dependent and stereoselective manner. Additionally, phencyclidine and other dissociative anaesthetics have been shown to be noncompetitive NMDA antagonists. In an effort to further probe the relationship between the PCP and NMDA receptors, the effects of PCP receptor acylation on AP7 behavioral responses were examined. Behavior was evaluated 24 hours after i.c.v. administration of metaphit in rats. The levels of metaphit which were routinely used (0.001-2.0 umole) induced neither ataxia nor stereotyped behavior when administered alone. However, metaphit, in a dose dependent manner, substantially enhanced the ability of AP7 (administered i.v. 24 hours after metaphit treatment) to induce these behaviors without significantly affecting their duration. Pretreatment of the rats with 0.5 µmole of metaphit lowered the ED_{50} for the D-AP7 induction of ataxia from 1.4 mmoles/kg to 0.4 mmoles/kg. NMDA receptor binding analysis on synaptic plasma membranes isolated from the forebrains of rats pretreated in vivo with metaphit revealed no alteration of Bmax values when compared to control membranes. Additionally, the Bmax and Kd for the NMDA receptor was unchanged following in vitro treatment of the synaptic plasma membranes with metaphit. This indicates that the metaphit potentiation of AP7 induced stereotyped behavior and ataxia is not due to an increase in NMDA receptor number. These results provide further evidence for the functional coupling of the NMDA and PCP recptors.

432.10 METAPHIT, AN ACYLATING PHENCYCLIDINE ANALOG, ENHANCES N-METHYL-D-ASPARTATE STIMULATED SODIUM FLUX. L.M. Pullan*, J.B. Monahan, R.C. Compton, W.F. Hood, T.L. O'Donohue, M.F. Rafferty[1], R.A. Lessor[1], K.C. Rice[1], and A.E. Jacobson[1] (SPON: P.C. Contreras). Searle Research & Development, CNS Diseases Research, St. Louis, Missouri and [1] National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland.

Tetrodotoxin insensitive ^{22}Na efflux from rat hippocampal slices is stimulated by excitatory amino acid agonists such as N-methyl-D-aspartate (NMDA). The stimulation of ^{22}Na efflux from preloaded hippocampal slices by NMDA can be blocked by the subclass selective antagonist, D-2-amino-5-phosphonovalerate (D-APV) and by phencyclidine (PCP). The antagonism by PCP is non-competitive while that of D-APV is competitive (pA_2=4.63). PCP and D-APV do not significantly affect the ^{22}Na efflux stimulated by kainate and α-amino-3-hydroxy-5-ethylisoxazole-4-propionic acid, agonists for other excitatory amino acid receptor subclasses. The PCP receptor acylating agent, metaphit, given to rats in vivo (lumole, icv), enhanced the NMDA stimulated ^{22}Na efflux assayed 24 hours later. The basal ^{22}Na flux was unaffected by metaphit pretreatment. The non-competitive inhibition by PCP and the enhancement by metaphit of the NMDA stimulated ^{22}Na efflux supports a close interrelationship of the PCP and NMDA receptors. It is possible that metaphit may allosterically enhance the probability of opening of the NMDA associated ion channel or that it may reverse a tonic inhibitory regulation by endopsychosin. The results also suggest that metaphit does not bind to the PCP site as an NMDA ion channel plug as has been postulated for PCP.

432.11 METAPHIT IRREVERSIBLY INHIBITS $CaCl_2$-DEPENDENT L-[^{3}H] GLUTAMATE BINDING. W.F. Hood[1], R.P. Compton[1]*, A.E. Jacobson[2]*, M.F. Rafferty[2]*, K.C. Rice[2]*, R.A. Lessor[2]* and J.B. Monahan[1] [1]Searle Research & Development, Chesterfield, Mo. 63198; [2]Laboratory of Chemistry, NIADDK, Bethesda, MD. 20205.

Evidence has been accumulating that indicates a strong functional coupling between the phencyclidine (PCP) and the excitatory amino acid receptors. PCP has been shown to inhibit the neurotransmission at certain excitatory amino acids synapses (N-methyl-D-aspartate preferring) and PCP binding has been shown to be enhanced by glutamate. In attempts to gain a further understanding of these interactions we tested the effect of a known PCP receptor acylating agent, metaphit, on glutamate binding.

We report that metaphit causes irreversible inhibition of $CaCl_2$-dependent L-[^{3}H]glutamate binding to rat synaptic plasma membranes (SPM). Preincubation of SPM with metaphit (50μM) for 5 minutes causes a 35% decrease in specific [^{3}H]glutamate binding. The decrease in binding is time and concentration dependent. Furthermore, the irreversible nature of metaphit's inhibition is apparent as after 3 washes of the SPM the decrease in binding remains. Also, if one treats rat forebrain homogenates (P2) with metaphit and then proceeds to isolate the SPM, the inhibition is still evident. This decrease appears to be selective for the 2-amino-4-phosphonobutanoate subclass of excitatory amino acid binding sites.

432.12 Locomotor hyperactivity induced by frontal cortex and intraventricular injections of PCP and the NMDA antagonist, AP-7. Katherine A. O'Neill and Jeffrey M. Liebman. Neuroscience Research, Pharmaceuticals Div., CIBA - GIEGY Corp., Summit, NJ 07901.

The distribution of phencyclidine (PCP) and n-methyl-d-aspartate (NMDA) receptors is similar (Monaghan and Cotman, J. Neurosci., 5, 1984; Contreras et al, Neurosci. Lett. 67, 1986), with high concentrations of both receptors in the frontal cortex. The NMDA antagonist, CPP (Harris et al, Br. Res., 382, 1986) produces hyperactivity upon injection into the frontal cortex (O'Neill and Liebman, Fed. Proc., 46, 952, 1987). Since NMDA and PCP receptors appear to be allosterically coupled (Loo et al, Eur. J. Pharmacol., 123, 1986), we compared the behavioral effects of the NMDA antagonist AP-7 with those of PCP after injection into the frontal cortex or lateral ventricle of rats.

Rats received injections of drugs in a saline vehicle through cannulae implanted into the right lateral ventricle, or bilaterally into the frontal cortex. Omni-tech activity monitors analyzed locomotion, and direct observations were also performed. Intra-ventricular injections of AP-7 (3,10 or 30 ug/5 ul) or PCP (30, 100 or 300 ug/ 5 ul) produced dose-related increases in distance traveled and movement time, accompanied by ataxia. In addition, PCP produced some sniffing behavior. In contrast, intra-frontal cortex AP-7 produced dose-related increases in distance traveled, movement time, vertical activity and perimeter walking. AP-7 treated rats showed a darting behavior similar to that seen after intra-frontal cortex CPP. No ataxia was observed. The effects of PCP in the frontal cortex were basically indistinguishable from those seen after intra-ventricular administration. These data show that the ataxic effects of AP-7 can be separated from the hyperactivity, while those of PCP cannot. The high doses required along with the apparent lack of anatomical specificity for PCP hyperactivity suggest that PCP may rapidly diffuse from the site of injection.

N-METHYL-D-ASPARTATE: PHYSIOLOGY

433.1 ANTICONVULSANT PROFILE OF AROMATIC ANALOGS OF ALPHA-AMINO OMEGA-PHOSPHONO CARBOXYLIC ACIDS. J.W. Ferkany, D.L. DeHaven, M.E. Guzewska*, R.L. Hudkins* and W.J. Rzeszotarski*. Divisions of Pharmacology and Chemistry, Nova Pharmaceutical Corporation, Baltimore, Maryland 21224-2788.

Antagonists of excitatory amino acids (EAA) may be useful to treat or prevent neurodegenerative disease, post-ischemic brain damage, epilepsy, anxiety and pain. The present study describes the anticonvulsant profile of a new series of aromatic analogs of alpha-amino omega-phosphono carboxcylic acids.

Compounds examined included the 4,5-benzo analogs of AP6 (NPC 532), AP7 (NPC 451) and AP8 (NPC 666) as well as 5,6-benzo AP7 (NPC 517) and 6,7-benzo AP8 (NPC 667). The potency of each compound to prevent chemically- and electrically-induced seizures (male CF-1 mice) was determined using standard methodologies and, toxicity assessed using the rotorod procedure. All compounds were administered ICV, with testing performed 15 min after injection. Inhibition of NMDA-evoked release of [^{14}C]acetylcholine from slices of rat corpus striatum was studied using the method of Lehmann et al. (J. Pharm. Exp. Ther., 232, 873, 1985).

NPC 517 and NPC 666 were found to be devoid of activity in the maximal electroshock (MES) and metrazol (PTZ) seizure models, whereas NPC 667 elicited convulsions when administered alone. Both NPC 451 (ED_{50} = 44 nmoles) and NPC 532 (ED_{50} = 91 nmoles) prevented PTZ- but not MES-induced seizures. In contrast, AP7 displayed anticonvulsant activity in both tests, having ED_{50} values of 6 and 36 nmoles in the PTZ and MES tests, respectively, whereas CPP was effective only in the MES procedure. Like AP7, NPC 451 prevented strychnine (STR)-, picrotoxin- and bicuculline (BIC)-induced seizures with a potency only slightly less than AP7. Both AP7 and NPC 451 prevented BIC- and STR-induced mortality in convulsing animals. With the rotorod test, NPC 451 (TD_{50} = 28 nmoles) had a more favorable TD_{50}/ED_{50} ratio than AP7 (TD_{50} = 1.1 nmoles), although both compounds were found to be toxic at anticonvulsant doses. NPC 451 was equipotent to AP7 in inhibiting NMDA-evoked [^{14}C]ACh release from perfused slices of corpus striatum.

The results of this study provide information on the structural requirements for the anticonvulsant effects of analogs of alpha-amino omega-phosphono carboxylic acids. Moreover, the data indicate that NPC 451 may be an NMDA receptor antagonist having a potency similar to that of AP7.

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433.2 RETARDATION OF AMYGDALA KINDLING BY ANTAGONISM OF N-M-D-ASPARTATE AND MUSCARINIC CHOLINERGIC RECEPTORS: EVIDENCE FOR THE SUMMATION OF EXCITATORY MECHANISMS IN KINDLING. D.P. Cain, K.A. Desborough and D.J. McKitrick. Dept. of Psychology, U. of Western Ontario, London, Ontario, CANADA N6A 5C2.

The role of the excitatory amino acids in epileptic and other phenomena is being intensively investigated. We have previously shown that an antagonist of N-m-D-aspartate receptors, DL-2-amino-5-phosphonovaleric acid (APV) significantly retards amygdala kindling (Soc. Neurosci. Abstr., 1986, 12, 69). In the present study we administered both APV and scopolamine hydrobromide (SCO), an antagonist of muscarinic cholinergic receptors, to determine the effect of blocking receptors of two major excitatory neurotransmitters implicated in amygdala kindling.

Male hooded rats received implantation of bipolar stimulating-recording electrodes in the basolateral amygdala and a guide cannula aimed at the cerebral ventricle. After determination of the AD threshold using 1-sec trains of 60 Hz biphasic square waves of increasing intensity, the rats received kindling stimulation once every 48 hr. APV-SCO rats received an icv injection of 42 ug APV in saline using an infusion pump, and an i.p. injection of SCO (10 mg/kg) 45 and 15 min, respectively, before each stimulation. Control rats received either saline alone or no injections. This continued for 15 sessions or until a stage 5 convulsion occurred, at which time, after a 7-d interval, the groups were crossed over to the opposite treatment and again stimulated after pretreatment until a stage 5 convulsion occurred.

After 15 sessions the APV-SCO group had reached a mean convulsion stage 2.2, and the control group reached a mean stage 4.9 ($p<.01$). Seven of 8 control rats kindled to stage 5, compared to 1 of 9 APV-SCO rats. The total mean number of ADs required by the APV-SCO rats to reach stage 5 was 25.2, compared to 12.0 in the control group ($p<.01$). These results indicate that the treatment with APV and SCO significantly retarded the rate of kindling in the APV-SCO group.

As the rate of kindling in the APV-SCO group (25.2 ADs) was slower than that in our previous groups treated with APV alone (17.3 ADs) or SCO alone (16.7 ADs), these data suggest that blocking two excitatory receptor types implicated in kindling leads to a greater retardation than blocking either type alone. This implicates a mechanism involving the summation of excitatory neurotransmission in amygdala kindling.

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433.3 NMDA RECEPTOR BLOCKER MK801 SUPPRESSES AMYGDALOID KINDLED SEIZURES IN THE RAT. C. Mack*, M.E. Gilbert, and R.S. Dyer (SPON: R. Janssen). Northrop Services, Inc. RTP, NC 27709 and U.S. EPA, Neurotoxicology Division, RTP, NC 27711.

MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclo hepten-5,10-amine) has been found to be highly potent in protection against maximal electroshock and bicuculline-induced seizures in mice (Clineschmidt, B.V. et al, Drug Dev. Res., 2:123-134, 1982). No data are available to evaluate the efficacy of this new NMDA receptor blocker (Wong,E. et al, Natl Acad Sci USA,83:7104-7108,1986) in a chronic seizure model. The ability of MK-801 to block seizures in amygdaloid kindled rats was investigated in the present experiment. Male Long Evans rats (N=6) were implanted with bipolar nichrome wire electrodes in the basolateral amygdala, and stimulated (1s train biphasic square waves at 60Hz) once daily until 3 to 4 generalized stage 5 seizures (rearing with bilateral forelimb clonus and loss of postural control) were observed. The following day, animals were administered 0.0, 1.0 or 10 mg/kg of MK-801 ip in physiological saline one-half hour before stimulation. All animals received all dosages in a counterbalanced fashion with a 72-hour interval between test days.

Behavioral responses to MK-801 prior to stimulation included enhanced motor activity and circling at the low dosage, and ataxia, paddling movements of the hindlimbs and headbobbing at the high dosage. The maximal seizure stage observed in animals receiving the high dose (10 mg/kg) of MK-801 was stage 3 (unilateral forelimb clonus contralateral to the side of stimulation). Five out of six subjects demonstrated only salivation and some flexion of the digits in response to stimulation. At 1.0 mg/kg, the maximal seizure stage attained was stage 4 (rearing with bilateral forelimb clonus), most subjects displaying only stage 3 seizures. All control animals responded with fully generalized stage 5 seizures. The duration of the evoked afterdischarge recorded in response to stimulation was decreased by MK-801 from a mean of 62 sec in the control to 36 sec in the low dosage (1.0 mg/kg) groups. Increasing the dose by a factor of 10 did not produce any further reduction in AD duration (mean=43 sec).

These findings demonstrate that MK-801 suppresses behavioral and electrographic seizures induced by electrical stimulation. Cain (Neurosci. Abs., 12:69,1986) reported similar effects with another NMDA-receptor blocker, APV. The anticonvulsant properties of MK-801 may depend on its antagonism of this receptor type.

433.4 BLOCKADE OF RAT CORTICAL SPONTANEOUS EPILEPTIFORM POTENTIALS WITH N-METHYL ASPARTATE ANTAGONISTS. D. Martin* and D. Lodge* (SPON: M.H. Bowman). Dept. Pharmacology, Duke Univ. Med. Ctr., Durham NC 27710 and Dept. Physiology, Royal Veterinary College, London NW1 OTU.

In several animal models of epilepsy, synaptic excitation mediated via N-methyl aspartate (NMA) receptors appears to play a key role in the genesis of epileptiform activity. It has also been shown that several groups of compounds, such as the phencyclidine/sigma opiate receptor ligands, divalent cations and omega phosphonates have the ability to reduce NMA-induced depolarisations. We have investigated the effects of a range of NMA antagonists on epileptiform discharge in wedges of rat cerebral cortex, specifically on the spontaneous potentials which develop upon removal of magnesium ions from the superfusion medium.

Grease seal techniques were used for recording responses from this preparation. Wedges (500um) comprising cerebral cortex and corpus callosum were prepared and perfused with magnesium-free oxygenated artificial cerebral spinal fluid until stable responses were obtained. Under these conditions, large negative-going potentials appeared spontaneously with up to 20 afterpotentials superimposed on the decay phase. Bath application of NMA antagonists for 60 min reduced both the frequency of spontaneous bursts and the number of afterpotentials in a dose dependent manner. IC_{50} values were estimated from data normalised as percentage of control activity.

	IC_{50}(uM)	
	Afterpotentials/burst	Bursts/min
Ketamine	1.5	3.9
±SKF 10,047	2.0	2.9
MK-801	0.086	0.14
DL-2-AP5	6.0	13.2
Mg++	170	220

The concentrations of the antagonists used in these studies are consistent with concentrations used to inhibit NMA-induced depolarisations. These results suggest that the spontaneous epileptiform potentials are mediated, in part, through the NMA receptor-ionophore complex. The grease seal technique may be useful in investigating the involvement of NMA receptors in epilepsy.

433.5 BLOCKADE OF NMDA RECEPTORS REDUCES PRESYNAPTIC AND POSTSYNAPTIC EXCITABILITY CHANGES DURING REPETITIVE STIMULATION OF CA1 PYRAMIDAL CELLS. N.P. Poolos and J.D. Kocsis. Depts. of Neurology, Stanford School of Medicine and Yale Medical School, and Palo Alto VA Medical Center, Palo Alto, CA 94304.

Several studies have shown that N-methyl-D-aspartate (NMDA) receptors in the hippocampus do not contribute to the postsynaptic response elicited by a single stimulus in the CA1 region of the hippocampus, but are activated during the high frequency stimulation used to induce long-term potentiation. We investigated the stimulation frequency-dependence of NMDA receptor activation and its contribution to changes in pre- and postsynaptic excitability during repetitive stimulation.

Extracellular stimulation and recording were performed in the rat hippocampal slice preparation. Trains of stimuli (5-80 Hz, 1 sec) were delivered in the stratum radiatum of CA1, and successive responses consisting of identifiable pre- and postsynaptic components were recorded. For each slice, trains were delivered in normal Krebs' and in solution containing 100 µM DL-2-amino-5-phosphonovaleric acid (APV), a selective blocker of NMDA receptors.

In normal solution, repetitive stimulation leads to a transient frequency-dependent facilitation of the postsynaptic response (as indicated by increases in the amplitude of the population spike and EPSP), followed by a progressive abolition of the postsynaptic response. Repetitive spiking is often seen during the facilitated phase. Blockade of NMDA receptors by APV significantly reduced activity-evoked changes in the postsynaptic response at all frequencies tested, including the occurrence of repetitive spiking. The reduction in facilitation was particularly evident at frequencies less than 40 Hz. These results demonstrate the presence of an NMDA receptor-mediated component of the postsynaptic response at frequencies as low as 5 Hz which contributes to facilitation of the postsynaptic response.

A similar attenuation of activity-evoked changes in the presynaptic response by APV was also observed. Since activity-evoked changes in presynaptic response have been found to correspond to changes in the extracellular potassium concentration ($[K^+]_o$), this suggests that blockade of NMDA receptors reduces increases in $[K^+]_o$ stemming from postsynaptic activity. Recordings obtained with K^+-sensitive microelectrodes have thus far confirmed this conclusion.

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433.6 GLYCINE DEPOLARIZES STRIATAL CHOLINERGIC INTERNEURONS INDEPENDENTLY OF THE NMDA-TYPE RECEPTOR. C.A. Taylor and J. Lehmann. Neuroscience/Cardiovascular Research, Pharmaceuticals Division, CIBA-GEIGY Corp., Summit, NJ 07901.

NMDA has been shown to evoke the release of [^{3}H]acetylcholine (ACh) from rat striatal slices (Lehmann and Scatton, Brain Res. 252: 77, 1982) Using the patch clamp technique, Johnson and Ascher recently reported that glycine potentiated the NMDA-evoked increase in the frequency of cationic channel openings (Nature 325: 529, 1987). In the present study we wanted to ascertain if a similar interaction existed between glycine and N-methyl-D-aspartate (NMDA)-type receptors mediating the release of [^{3}H]ACh from rat striatal slices.

[^{3}H]ACh release evoked by NMDA (50uM) was decreased 90% by 1.2 mM $MgCl_2$, 70% by the competitive NMDA-type receptor antagonist CGS 19755 (10 uM), 50% by 10 nM MK801, a compound which blocks NMDA-evoked responses, and 70% by 1 uM strychnine. Release of [^{3}H]ACh evoked by 15 mM KCl was not blocked by CGS 19755, MK801 or strychnine. KCl-evoked release was decreased 60% by 1.2 mM $MgCl_2$.

The release of [3H]ACh was also evoked by 100 uM glycine. This effect was not blocked by $MgCl_2$, CGS 19755 or MK801. However, the glycine-evoked release was decreased 90% by 1 uM strychnine. Glycine (100 uM) did not produce a cross-tachyphylaxis to NMDA. Moreover, 100 uM glycine failed to potentiate the effects of 50 uM NMDA on [^{3}H]ACh release.

The lack of an interaction between glycine and the NMDA-type receptor-mediated response measured here differs from that described in single channel recording from cultured mouse neurons by Johnson and Ascher. In the present study, glycine apparently produces a depolarization of striatal cholinergic neurons which is independent of NMDA-type receptors. In contrast, in the patch clamp studies, glycine did not exert an effect on its own but potentiated the effect of NMDA. Moreover, in the present study, the effect of glycine on the striatal cholinergic neuron appears to be sensitive to strychnine while in patch clamp studies strychnine failed to block the action of glycine. The present data show that the strychnine-sensitive glycine-evoked release of [^{3}H]ACh from rat striatal slices is not mediated via an NMDA-type receptor.

433.7 INTERACTIONS OF NICOTINIC CHOLINERGIC DRUGS WITH N-METHYL-D-ASPARTATE (NMDA)-INDUCED HIPPOCAMPAL ^{3}H-NOREPINEPHRINE RELEASE. S.M. Jones, L.D. Snell* and K.M. Johnson. Dept. Pharmacol. and Toxicol., Univ. Texas Med. Branch, Galveston, TX 77550.

We have reported (JPET 240:492, 1987) that the glutamate analogues N-methyl-D-aspartate (NMDA) and kainate (KA) can stimulate ^{3}H-norepinephrine (NE) release from superfused rat hippocampal slices. To gain a better understanding of the interactions of transmitter systems in the hippocampus, we examined the effects of cholinergic drugs on spontaneous and NMDA-induced ^{3}H-NE release.

Rat hippocampal slices (0.4mm) were incubated with 50 nM ^{3}H-NE (30 min; 37°C), rinsed and placed into superfusion chambers assembled in a 37°C water bath. Because NMDA-induced NE release is strongly inhibited by magnesium ions, slices were superfused in magnesium-free Kreb's buffer. After a 60 min washout period, superfusate was collected in 10 three-min fractions. The effects of cholinergic drugs were estimated by subtracting the release during the first three fractions from the release in the presence of drug over the next three fractions. NMDA-induced release was calculated by subtracting the release in these drug containing fractions from that seen in the fractions following an NMDA pulse (100 uM; 2 min).

The nicotinic agonists dimethylphenylpiperazinium (DMPP) and carbachol (CARB), as well as nicotine (NIC) itself all produced concentration dependent increases in ^{3}H-NE release (DMPP >> NIC > CARB). The enhanced release produced by NIC and CARB but not by DMPP showed an apparent desensitization and all three could be antagonized by the non-competitive nicotinic antagonist mecamylamine (MECA) and the competitive antagonist hexamethonium (C6). DMPP and CARB, but not NIC enhanced NMDA-induced NE release in a manner that was not merely additive with their effects on spontaneous release. The effects of DMPP and CARB on NMDA-induced release were reduced by MECA but not C6. The muscarinic agonist bethanecol and the muscarinic antagonist atropine did not alter spontaneous or NMDA-induced NE release.

Surprisingly, MECA alone was found to reduce NMDA- but not KA-induced release. The selective inhibition of NMDA-induced release is a characteristic of phencyclidine (PCP)-like drugs. Subsequently we found that MECA and pempidine (PEMP), a tertiary amine with similar ganglionic blocking properties, can displace ^{3}H-TCP from homogenates of rat hippocampus, but that C6 does not. Although the IC_{50} values of MECA and PEMP (10 uM and 22 uM, respectively) are considerably higher than PCP (IC_{50}=46 nM) these concentrations are well within the range used to antagonize nicotinic responses. These data demonstrate an interaction between excitatory nicotinic and amino acid receptors on noradrenergic terminals in the hippocampus. Further, non-competitive ganglionic blockers may exert effects via the PCP/sigma receptor. Supported by DA-02073.

433.8 FACILITATION OF NMDA-EVOKED VENTRAL ROOT DEPOLARIZATIONS AND POTASSIUM RELEASE AFTER EXPOSURE TO NMDA ANTAGONISTS IN THE ISOLATED FROG SPINAL CORD. D.X. Zhang*, J.C. Hackman, J.L. Vega*, A.M. Holohean*, and R.A. Davidoff. Department of Neurology, University of Miami School of Medicine and Neurophysiology Laboratory, VA Medical Center, Miami, FL 33101

Excitatory amino acid receptors have been divided into three types based upon activation by specific agonists. However, specific antagonists [e.g., 2-amino-5-phosphonovaleric acid (APV)] are available for only one type of receptor, the N-methyl-D-aspartate (NMDA) receptor. Data from the present study show that following application of APV, NMDA-evoked motoneuron depolarizations and changes in K^+ levels were larger than control responses.

Frogs (Rana pipiens) were anesthetized on ice, decapitated, and the spinal cord with the attached 9th and 10th dorsal and ventral roots (VRs) removed. The isolated cord was placed in a sucrose gap chamber to differentially record VR DC membrane potentials. K^+-sensitive microelectrodes were used to measure changes in extracellular K^+ concentrations ($[K^+]_o$) in the dorsal horn and intermediate gray matter. Cords were superfused with HCO_3^--Ringer's solution bubbled with 95%O_2:5%CO_2 and maintained at 15°C. Addition of NMDA (30-300 uM, 10 sec) to the medium depolarized the VR (2-6 mV) and elevated $[K^+]_o$ (0.5-8 mM). Addition of APV (3-30 uM) to the Ringer's solution resulted in block of spontaneous VR activity, hyperpolarization of the VR (0.32-3.0 mV), decrease of resting $[K^+]_o$ (0.12±0.03, n = 4), and block of NMDA-induced VR depolarizations. After one hour applications of APV and two hours of washing with Ringer's solution, VR potential changes produced by 30-100 uM concentrations of NMDA returned to control levels, but VR depolarizations produced by 300 uM NMDA increased 33.5 ±8.9% ($p < 0.05$) compared to control levels. In addition, NMDA-evoked $[K^+]_o$ was increased (12%). The enhanced response was also present in cords bathed in medium containing 0.625 uM TTX. After APV applications no changes were observed in quisqualate-induced VR depolarizations or in quisqualate-induced changes in $[K^+]_o$.

In summary, blockade of NMDA receptors with APV resulted in an increase in VR depolarizations and potassium release evoked by high concentrations of NMDA. The response appeared to be specific for NMDA receptors. (Supported by VA MRIS #1769 and 3369 and USPHS #17577)

433.9 NMDA EFFECTS ON NEURONS IN NEOCORTICAL EXPLANT CULTURES. B. Wolfson and F. Baldino, Jr., E. I. du Pont de Nemours and Co., Wilmington, DE 19898

We have previously shown that explanted neocortical neurons organize into circuits which can spontaneously initiate epileptiform activity or support synchronous discharge induced by epileptogenic drugs. Activation of NMDA receptors contributes to this synchronous discharge which can even occur in the presence of inhibitory synaptic transmission. In this study, we examined the responses of explanted neocortical neurons to iontophoretically applied NMDA.

Explants of parietal cortex were prepared from newborn rats and maintained 3-5 weeks in flying-coverslip rollertubes. Intracellular recordings were performed with high resistance micropipettes placed visually with the aid of an inverted phase-contrast microscope. Pharmacological agents were applied iontophoretically or through the superperfusate.

Most neurons responded to NMDA with voltage-dependent depolarizations accompanied by spiking and an increase in conductance. Often, application of NMDA led to prolonged oscillations of voltage during which fast spikes arose from potentials subthreshold for directly elicited spikes. Presumably, these lower threshold spikes originated from regions electronically remote from the site of impalement. Frequently, NMDA elicited slow depolarizations upon which trains of fast spikes or repetitive wavelets occurred. Varying degrees of desensitization to NMDA were observed with repetitive application. Preliminary observations suggest desensitization to NMDA may also be voltage-dependent. All responses to NMDA were blocked by the NMDA receptor antagonist, 2-aminophosphonovalerate (APV). Occasionally, APV-sensitive spontaneous activity that mimicked responses to applied NMDA were observed.

These observations suggest that NMDA may function as a transmitter in neocortical explant cultures but that desensitization limits the contribution of this amino acid to recurrent excitation. Some features of neuronal responses to NMDA suggest that they arise in remote regions of the cell such as the dendrites. If regenerative, voltage-dependent responses are initiated in dendrites, remote excitatory input could lead to significant depolarization at the soma. This may be one mechanism which contributes to spontaneous synchronous discharge in this preparation.

433.10 EVIDENCE OF A ROLE FOR CORTICAL N-METHYL D-ASPARTATE (NMDA) RECEPTORS IN DELTA WAVE SLEEP. K. Fox* and M. Armstrong-James* (SPON: W.J. Brunken) Dept. Physiology, Turner St. London E1 2AD, U.K.

During delta-wave, or stage 3-4, sleep neurons in layer V of the rat cortex tend to fire together in bursts of spikes interrupted by intermittent periods of neuronal silence. These bursts recur at a frequency of 0.5-4 Hz in contrast with the 8-14 Hz frequency of spindles which characterize stage 2 sleep. Delta sleep bursts were found to commence synchronously (50% within 40-80ms) over widespread areas of frontal and parietal cortex bilaterally. Similar burst-pause activity occurs in the cortex of rats anesthetized with urethane and has been shown to depend on the activation of an NMDA receptor. The NMDA antagonist amino phosphono valeric acid (APV) might therefore be expected to inhibit spontaneous burst-pause activity during stage 3-4 sleep. This was investigated by chronically implanting carbon fibre iontophoretic electrodes in rats under halothane anaesthesia and iontophoresing APV or NMDA during natural sleep epochs after recovery. A second electrode located in the cortex of the opposite hemisphere allowed continuous monitoring of focal EEG and pattern of spike activity during iontophoretic trials. APV was found to decrease the duration of bursts during stage 3-4 sleep and decrease their probability of occurrence on 8 cells (35/35 trials; current range 10-50 nA), recovery occurring within 60-100 seconds. APV was ineffective on 3 cells. Sleeping animals often alternated between stage 3-4 and stage 2 sleep. When this occurred during an APV trial it was found that, despite elimination of bursts, neuronal spindle activity was unaffected. APV was also ineffective in inhibiting responses to cutaneous stimulation during quiet waking.

A total of 14 NMDA trials were conducted on eight cells during stage 3-4 sleep causing excitation in each case (current range 5-30 nA). NMDA acted to increase the number of spikes per burst principally within the first second of each burst. On 3 occasions NMDA was iontophoresed during quiet waking at currents of 10-25nA, causing tonic excitation. At no time was NMDA found to induce burst-pause activity in neurones during quiet waking at currents sufficient to produce a 50% increase in firing rate.

The mutual susceptibility of bursts to inhibition by APV during stage 3-4 sleep and urethane anaesthesia further indicates the similarity of spontaneous activity in the two states. Previous work in urethane anaesthetised animals has suggested that bursts are driven in the cortex by the spontaneous activity of the anterior intralaminar nuclei which act via a cortical NMDA receptor. The intralaminar nuclei may prove to be acting in a similar way during stage 3-4 sleep to cause cortical burst-pause activity. These results indicate that NMDA receptors are involved in the generation of bursts characteristic of delta wave sleep but not spindles which occur during stage 2 sleep.

433.11 SEROTONIN ENHANCES RESPONSES TO N-METHYL-D-ASPARATE IN RAT NEOCORTICAL NEURONS. J.N. Reynolds*, A. Baskys and P.L. Carlen. (SPON: G.A. Lnenicka), Playfair Neuroscience Unit, Toronto Western Hospital, Addiction Research Foundation, and the Departments of Physiology and Medicine, University of Toronto, Toronto, Ontario Canada.

Serotonergic axons are distributed with a highly uniform pattern throughout the neocortex of the rat. Preliminary experiments showed that serotonin (5-HT) induced a concentration-dependent increase in the extracellular field potential in neocortical slices. Therefore, we examined the possible interaction between 5-HT and the excitatory amino acid N-methyl-D-aspartate (NMDA) in slices of the rat neocortex.

Coronal slices (400um) of the fronto-parietal cortex of the Fischer 344 rats (4-6 months of age) were cut using a vibratome. Slices were perfused with a medium containing (mM): NaCl (124), KCl (3), KH_2PO_4 (1.25), $MgSO_4$ (2), $CaCl_2$ (2), $NaHCO_3$ (26), dextrose (10), aerated with 95% O_2-5% CO_2, pH 7.4. Drugs were applied by perfusion or pressure ejection. Intra and extracellular recordings were obtained in layers IV/V using conventional techniques. Bipolar tungsten stimulating electrodes were placed at the boundary of the cortical white matter and layer VI for afferent stimulation.

In 75% of cells examined with intracellular recordings, perfusion with 10uM 5-HT did not change resting membrane potential (RMP), input resistance (Rin) or spike threshold as determined by intracellular current injection. In a few cells, 5-HT produced a 2-3 mV hyperpolarization with an accompanying decrease in Rin. The hyperpolarization was reversible by washing. In 50% of cells tested, 5-HT produced a long-lasting increase in the EPSP amplitude. Other cells showed either no change (25%) or a decrease (25%). NMDA (0.5mM) was pressure-ejected approximately 50um dorsally from the recording site, causing a depolarization with increased Rin. After perfusion with 10uM 5-HT, these cells exhibited an increased response to NMDA. The duration of the depolarizing response was doubled, and there was an increase in the frequency of action potentials generated by NMDA. This effect was long-lasting, and could not be reversed by washing the tissue for up to 60 minutes.

It is suggested that 5-HT increases the sensitivity of NMDA receptors for the agonist. This effect is long-lasting, and indicates a neuromodulatory role for serotonin.

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433.12 NMDA- AND NON-NMDA-MEDIATED COMPONENTS OF SYNAPTICALLY EVOKED INPUT FROM LAYER 2/3 CELLS TO PYRAMIDAL CELLS IN LAYER 5 OF RAT VISUAL CORTEX. Kenneth A. Jones and Robert W. Baughman. Harvard Medical School, Dept. of Neurobiology, Boston, MA.

Excitatory neurotransmission in cortical structures is thought to involve primarily glutamate receptors. In the hippocampus synaptic excitation appears to be mediated by both NMDA and non-NMDA receptor subtypes. The pharmacology of glutamate neurotransmission in the neocortex is less well understood. One type of synaptic response mediated by NMDA receptors can be elicited by stimulating the underlying white matter (Thompson et al, 1986), but the presynaptic source of this response is not known. In the present study the pharmacological properties of intracortical synapses were examined by intracellularly recording epsp's in cells in layer 5 while electrically stimulating layer 2/3. All of the layer 5 neurons were pyramidal in nature as determined by injections of Lucifer Yellow or by electrophysiological criteria. The stimuli never produced direct activation of layer 5 cells, which supports the interpretation that layer 2/3 cells are the source of the observed epsp's. Epsp's generated in the layer 5 cells by low intensity stimuli to layer 2/3 occurred with a short (2.0 - 2.5 ms) and constant latency, and appeared not to be contaminated with polysynaptic components. Application of kynurenic acid (KA) to the bathing medium reversibly antagonized the epsp in a dose dependent manner: 1 mM (n=4) and 5 mM (n=6) KA produced 63% and 79% reductions respectively of the control response. Gamma-D-glutamylglycine (γ-DGG) at 1 mM (n=3) caused a comparable degree of antagonism while GDEE at 1 mM (n=3) had no effect. These results are consistent with the pharmacology of synapses made on identified layer 5 cells in culture (Huettner and Baughman, 1986), and support the idea that layer 2/3 pyramidal cells make glutamatergic/aspartergic synapses with pyramidal cells in layer 5.

Since the effective concentrations of KA and γ-DGG were high enough to act on NMDA as well as non-NMDA receptors, we tested the effectiveness of the specific NMDA antagonist APV in the presence and absence of Mg^{++}. When applied to slices bathed in 1 mM Mg^{++}, APV (50-100 μM) caused a small reduction (10-20%) in peak amplitude and duration of the falling phase of the epsp in 9 out of 10 neurons tested. When slices were bathed in medium containing 0 mM Mg^{++} the amplitude of the epsp increased substantially. Under these conditions APV reduced the size of the epsp to that observed with APV in the presence of 1 mM Mg^{++} (n=2). The voltage dependence of the epsp in the presence of 1 mM Mg^{++} was determined by depolarizing and hyperpolarizing the cell while stimulating layer 2/3. For all cells (n=6) the APV sensitive component of the epsp was greatest at the most positive potential tested (-45 mV) and nearly absent at membrane potentials below rest. The APV-sensitive component at -45 mV ranged from 15-60% of the total response. Although the latency of the APV-sensitive component was only 1-2 msec longer than that of the APV-insensitive one, the time to peak was 3-10 msec longer. The long time to peak for the NMDA response has been demonstrated in other systems and appears to be characteristic of this receptor subtype.

These results provide evidence that the layer 2/3 to 5 pathway, which comprises a major interlaminar circuit in cortex, is mediated directly through NMDA as well as non-NMDA receptors. This finding has implications for the role of this circuit in cortical visual plasticity. (NIH EY03502 and EY05810)

433.13 ANTITUSSIVE AGENTS, DEXTRORPHAN AND DEXTROMETHORPHAN ANTAGONIZE N-METHYL-D-ASPARTATE AND ARE ANTIEPILEPTIC. B.Y. Wong*, D.A. Coulter*, D.W. Choi, and D.A. Prince (SPON: M.E. Smith). Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Antagonists to the N-methyl-D-aspartate (NMDA) receptor have anticonvulsant properties in several models of experimental epilepsy. It has recently been reported that sigma opiates antagonize NMDA, possibly via a common receptor. Among this "sigma" group of drugs is the common cough suppressant dextromethorphan (DM) and its metabolite dextrorphan (DX). We therefore tested DM and DX for anticonvulsant properties in vitro, in guinea pig neocortical brain slices.

Vibratomed sections of sensorimotor cortex were maintained in an interface type chamber at 37°C in normal oxygenated Ringer's. Prolonged ictal afterdischarges and interictal bursts were induced by perfusion with solution free of Mg^{++} and recorded extracellularly. These pathological activities were blocked by both DX (1-100 μM bath; 250 μM topically) and DM (100 μM bath). The competitive NMDA antagonist, D-L-amino-phosphono-valerate (APV) also blocked by epileptiform discharges (50 μM bath; 250 μM topically). Intracellular records showed that these drugs block NMDA-induced depolarizations without altering intrinsic membrane properties (Coulter and Prince, Neurosci. Abstr., 1987). Extracellular multiple unit recordings showed that DX and DM blocked NMDA but not quisqualate-induced excitatory responses. Glutamate responses were partially suppressed by DX and DM. The time course of DX or DM action on epileptiform activity or NMDA responses was much longer than for APV; APV effects were washed out in minutes whereas DX or DM suppressed epileptiform activity for hours.

As NMDA antagonists, these drugs represent a potentially new class of anticonvulsant for human use. Given the wide availability and established clinical experience with DM, and its experimental effectiveness as an anticonvulsant agent, we suggest that it should be expeditiously tested in human epilepsy.

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433.14 DEXTROMETHORPHAN AND DEXTRORPHAN ANTAGONIZE DEPOLARIZING RESPONSES OF NEOCORTICAL NEURONS TO EXCITATORY AMINO ACIDS. D.A. Coulter* and D.A. Prince. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Recent evidence suggests that blockade of excitatory amino acid receptors depresses epileptogenesis in a variety of animal models, and may also protect cortical neurons from hypoxic damage. Dextromethorphan (DM), an agent found in nonprescription antitussives, is reported to block extracellularly recorded responses to N-methyl-D-aspartate (NMDA) in spinal cord (Church et al., Eur. J. Pharm. 111: 185, 1985). In the present and a companion study (Wong et al., Neurosci. Abstr. 1987), we examined the potential actions of DM, and its active metabolite, dextrorphan (DX), as excitatory amino acid antagonists and anticonvulsants.

Slices of guinea pig cingulate and sensorimotor cortex were prepared using conventional methods. Intracellular recordings from layers II-V were obtained using 4 M K acetate-filled electrodes. Glutamate, NMDA, quisqualate, and kainic acid were applied focally either by brief pressure pulses to a broken micropipette, or by iontophoresis. Control responses and those obtained in the presence of focally applied DM (1 mM) or DX (200 μM) were compared. All of the above excitatory amino acids depolarized cortical neurons. Kainate, quisqualate, and (for the most part) glutamate depolarizations were accompanied by increases in conductance. NMDA-induced depolarizations of small magnitude were often accompanied by apparent decreases in membrane conductance (as occasionally were small glutamate depolarizations). This conductance decrease persisted when the membrane potential was manually clamped to the resting level during NMDA application. Larger applications of NMDA sometimes resulted in 'bistable' oscillations of membrane potential between two levels, 10-20 mV apart. Puff application of DM or DX reversibly blocked NMDA-induced depolarizations, and, in some cases, glutamate-induced depolarizations, but not kainate or quisqualate depolarizations elicited in the same cells. This antagonist effect was often long-lasting, requiring 30-40 minutes to reverse. DM would often reduce the size of EPSPs elicited by white matter stimulation by 50-70%. No effect of either DM or DX on input resistance, membrane potential, or spike height of cortical neurons was noted. Our data suggest that DM and DX act as specific NMDA antagonists on cortical neurons. These agents may be useful in the treatment of epilepsy and potential hypoxic injury. Their availability and apparent nontoxicity would argue for an expeditious clinical trial.

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433.15 NMDA RECEPTORS PARTLY MEDIATE L-PROLINE-INDUCED DEPOLARIZATION OF RAT SPINAL MOTONEURONES. B. Ault*, B.C. Yawn* and C.M. Wang. Laboratory of Biophysics, Dept. of Pharmacology, Burroughs Wellcome Co., Research Triangle Park, NC 27709, U.S.A.

It has been reported previously that L-proline (L-pro) excites hippocampal pyramidal cells by an action at acidic amino acid receptors (Ault & Nadler, Soc. Neurosci. Abstr., 10, 229, 1985). We have more directly investigated this effect by examining the action of L-pro in the isolated spinal cord of the neonatal rat.

Spinal cords were dissected from 3-6 day old rats and hemisected by cutting down the ventral fissure. The preparations were maintained between 25 and 26°C and superfused at 1 ml/min with medium of composition (mM): NaCl, 118; KCl, 3; $NaHCO_3$, 24; $CaCl_2$, 2.5; NaH_2PO_4, 2.1; D-glucose, 12, which was gassed with 95% O_2/5% CO_2. The L4 or L5 dorsal root was mounted on a stimulating electrode and d.c. recordings were made from the corresponding ventral root. Agonists were applied in 2 ml pulses while antagonists were present continuously in the medium.

L-pro (1-8 mM) depolarized ventral roots of spinal cords superfused with standard medium, or in the presence of tetrodotoxin (10^{-7} M), in a dose-dependent manner with one sixth of the potency of L-glutamate (L-glu). L-pro was four times more potent than D-pro. Other substituted analogs of L-pro also had reduced potency. Depolarizations induced by L-pro showed no apparent desensitization. Application of L-glu prior to, during, and following prolonged superfusion of L-pro provided no evidence of a partial agonist action of L-pro. Ca^{++}-free medium potentiated the depolarizing action of L-pro, L-glu and quisqualate. L-pro was antagonized by concentrations of 2-amino-5-phosphonovalerate (25 µM), Mg^{++} ions (1 mM) and γ-D-glutamylglycine (0.1 mM) that depressed N-methyl-D-aspartate (NMDA)-induced depolarizations. The NMDA receptor-mediated component of the L-pro-induced response was estimated to be 60-70%. These data are consistent with reports of high affinity proline uptake and potassium-induced release that suggest a neurotransmitter role for L-pro. Since L-pro is a neutral compound, our observations raise the possibility that acidic amino acid receptors may not require an agonist to possess two anionic groups and one cationic group.

433.16 NMDA DISCRIMINATIVE STIMULI ARE ANTAGONIZED BY COMPETITIVE NMDA ANTAGONISTS, PCP-TYPE COMPOUNDS AND KAPPA AGONISTS. C.L. Amrick and D.A. Bennett. Neuroscience Research, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, N.J. 07901.

It has recently been reported that a subconvulsive dose of the excitatory amino acid N-methyl-D-aspartate (NMDA, 30 mg/kg i.p.) produces discriminative stimuli in male Sprague Dawley (Crl:CDBR) rats (Amrick and Bennett, Life Sci. 40:585, 1987). The present experiments evaluated four classes of compounds for their ability to block this discriminative cue: competitive antagonists at the NMDA receptor, PCP-type drugs, sigma-type drugs and opiates (including kappa and mu agonists).

3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) (Amrick and Bennett, ibid) and CGS 19755, two potent and competitive NMDA antagonists, dose-dependently blocked NMDA discriminative stimuli with ED50 values of 2.0 and 0.18 mg/kg i.p., respectively (for the pharmacological profile of CGS 19755, see Bennett et al., and Lehmann et al., this meeting).

Since NMDA modulation has been associated with PCP-type compounds (Anis et al., Br. J. Pharmacol. 79:565, 1983), both PCP and sigma compounds were assessed in this model. It has been previously reported that both dexoxadrol (ED50 = 0.73 mg/kg i.p.) and ketamine (ED50 = 4.16 mg/kg i.p.) antagonized these NMDA stimuli (Amrick et al., Pharmacol. 28:114, 1986). In the present experiments, PCP also produced dose-dependent antagonism (ED50 = 0.22 mg/kg i.p.). These potent effects of PCP-type compounds contrast with results obtained with sigma agonists. (+)-SKF 10,047 produced a maximum of 50% antagonism from doses of 1 to 10 mg/kg i.p. Lower doses produced less effect, while higher doses produced severe response suppression. The sigma-active isomer, (+)-SKF 10,047, also produced a 50% effect in the same dose range, while (-)-SKF 10,047 produced dose-dependent antagonism (ED50 = 1.45 mg/kg i.p.). Testing with isomers of 3-PPP also indicated that the sigma-active isomer ((+)-3-PPP) produced no antagonism of the cue in doses from 1 to 10 mg/kg i.p., but that antagonism was produced by (-)-3-PPP (ED50 = 0.94 mg/kg i.p.).

Among the opiates tested, the selective kappa agonists, tifluadom (ED50 = 0.15 mg/kg i.p.), ethyl-keto-cyclazocine (ED50 = 0.19 mg/kg i.p.) and U50,488H (ED50 = 0.94 mg/kg i.p.), each dose-dependently blocked the cue in a low dose range. Nonselective kappa agonists having mu agonist/antagonist or sigma activity (i.e., cyclazocine and pentazocine), as well as morphine, had little to no effect even up to response-suppressing doses.

These data indicate that competitive antagonists at the NMDA receptor, as well as PCP-type compounds and kappa agonists, antagonize the discriminative stimuli produced by a subconvulsive dose of NMDA. The lack of effectiveness of sigma-active compounds suggests that this assay may distinguish between PCP-active and sigma-active compounds.

433.17 THE BEHAVIORAL PHARMACOLOGICAL PROFILE OF AN N-METHYL-D-ASPARTATE (NMDA) ANTAGONIST, CGS 19755. D.A. Bennett, P.S. Bernard*, C.L. Amrick, D.E. Wilson* and A.J. Hutchison*. Neuroscience Research, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, N.J. 07901.

CGS 19755 (cis-4-phosphonomethyl-2-piperidine-carboxylic acid) is a competitive antagonist of the excitatory amino acid receptor responsive to N-methyl-D-aspartate (NMDA) (Lehmann et al., this meeting). The compound potently antagonized NMDA (154 mg/kg i.p.)-induced convulsions when administered either intraperitoneally (ED50s ranging from 2.3 to 48 mg/kg with a 0.5 to 8 hour pretreatment) or intravenously (ED50s ranging from 0.9 to 1.7 mg/kg with a 5 to 30 min pretreatment) to mice (Crl:CF1BR). CGS 19755 (MED = 3 mg/kg i.p.) also significantly increased the mouse convulsion threshold of i.v. administered NMDA.

This NMDA antagonist produced an increase in conflict responding of 459% at 1.7 mg/kg i.p. in rats (Crl:(WI)BR). An attenuation of this increased response was noted with higher doses of the compound, presumably due to the onset of overall response suppression (decreases in non-conflict responding). In support, loss of traction (i.e. muscle relaxation/sedation) was also noted in mice, albeit at doses higher than those which induced anticonvulsant effects (ED50s for traction loss range from 7 to 74 mg/kg i.p. with a 0.5 to 8 hour pretreatment). In rats, CGS 19755 produced rotorod deficit with an ED50 value of 6 mg/kg i.p.

CGS 19755 generalized to diazepam-induced discriminative stimuli in rats (Crl:CDBR) (ED50 = 9 mg/kg i.p.). In rats trained to discriminate NMDA from saline (Amrick and Bennett, Life Sci. 40:585, 1987), CGS 19755 produced potent antagonism of the cue at low doses (ED50 = 0.1 mg/kg i.p.) and generalized to the discriminative stimuli at higher doses (ED50 = 6.7 mg/kg i.p.). These latter data suggest that CGS 19755 produces properties similar to NMDA at higher doses, although the compound does not produce NMDA-like effects in other test systems (i.e., CGS 19755 is not convulsant, etc.).

CGS 19755 produced a motor activity syndrome in mice similar to PCP (increased movement time, distance traveled and perimeter walking) at 30 mg/kg i.p., substantially above the anticonvulsant and anticonflict doses. This effect showed a delayed onset beginning at approximately 90 min post dosing.

These data indicate that CGS 19755 is a potent NMDA antagonist in vivo. It produces anticonvulsant and anticonflict effects supporting the contention that NMDA antagonists, as a class, may be therapeutically effective anticonvulsants and antianxiety agents.

433.18 EVIDENCE SUGGESTING DIFFERENTIAL EFFECTS OF N-METHYL-D-ASPARTIC ACID (NMDA) AND L-HOMOCYSTEIC ACID (LH) ON CEREBELLAR PURKINJE NEURONS. M. Lee, H.K. Strahlendorf, R.L. Cooper*, and J.C. Strahlendorf. Depts. of Physiology and Neurology, Texas Tech Univ. Hlth. Sci. Ctr., Sch. of Med., Lubbock, Texas 79430.

A recent study demonstrated a potassium-induced, calcium-dependent release of endogenous homocysteic acid from rat brain (J. of Neurosci. 6: 2226-2234, 1986). Using intracellular recording techniques, they also reported that microiontophoretic applications of LH induced depolarization similar to that induced by NMDA, and both depolarizations were antagonized selectively by the specific NMDA antagonist, DL-2-amino-7-phosphonoheptanoic acid (APH) in the caudate nucleus. Olney and his colleagues (Soc. for Neurosci. Abstr. p380, 1986) also found that LH causes a lesion with the same cellular distribution and ionic mechanisms as a NMDA lesion. On the basis of these studies, the above investigators suggested that LH may be a natural transmitter at NMDA receptor sites. Considering similar actions of NMDA and LH, we examined the effects of NMDA and LH on cerebellar Purkinje cells and compared the interactions of these amino acids with various excitatory amino acid antagonists.

In urethane anesthetized rats, iontophoretic application of NMDA elicited three different effects on the spontaneous activity of Purkinje cells during and after the drug ejection period: excitation, inhibition, and biphasic responses consisting of excitation followed by inhibition. On the other hand, LH elicited excitation, only, regardless of the actions of NMDA on the same neurons. When the effects of various excitatory amino acid antagonists were examined on NMDA- and LH-mediated responses, excitatory effects of NMDA were antagonized effectively by dl-2-amino-5-phosphonovalerate (APV), ketamine, γ-d-glutamylglycine (DGG), and APH and were not influenced significantly by l-glutamate diethylester (GDEE). Inhibitory responses of NMDA were antagonized by APV, APH, and ketamine. LH-mediated excitations were influenced most by DGG and to successively lesser degrees by ketamine, GDEE, APV, and by APH. Thus, effects of LH were attenuated least by specific NMDA antagonists. These findings indicate that major actions of LH may not be mediated through NMDA receptor sites, at least in the cerebellum. (Supported by NIH Grant RO1 NS 19296).

434.1 GLUTAMATE-LIKE IMMUNOREACTIVITY (Glu-LI) IN PUTATIVE GLUTAMATERGIC NERVE ENDINGS: ACCESSIBILITY OF ANTIGENS TO ANTIBODIES.
J. Storm-Mathisen* and O.P. Ottersen (SPON: ENA). Anatomical Institute, University of Oslo, Karl Johansgt. 47, N-0162 Oslo 1, Norway.

Glutamate (Glu) and other amino acids can be demonstrated immunocytochemically in tissue fixed with glutaraldehyde (Storm-Mathisen et al., Nature, 301: 517-520, 1983). Previously we have found evidence that "transmitter Glu" can be preferentially revealed in brain slices kept in isotonic media in vitro prior to fixation and that the Glu-LI is preferentially localized in putative glutamatergic nerve endings in slices of hippocampus (Storm-Mathisen et al., Med. Biol. 64: 127-132, 1986). However, in "standard" light microscopic preparations from animals fixed by perfusion with glutaraldehyde Glu-LI is generally low in nerve endings (Ottersen and Storm-Mathisen, Neuroscience, 16: 589-606, 1985). The experiments reported here address the question of what is the "true" in vivo localization of Glu.

Rats were fixed by rapid transcardial perfusion with 5% glutaraldehyde in 0.1M NaPi pH 7.4. Frozen sections were mounted on subbed slides but not allowed to dry. They were then processed with Glu antibody no. 13 (PAP procedure as described before), with or without preincubation in trypsin or chymotrypsin (1mg/ml, 100mM Tris/HCl, pH 8, 15-45 min, 25°C). The proteolytic treatment caused an increase i Glu-LI in the neuropil of hippocampus corresponding to the zones of termination of putative glutamatergic pathways, notably CA3 and CA4 pyramidal axons; terminals of mossy fibres were clearly stained. As a rule staining of pyramidal and granular cell bodies and main dendrites were concomitantly reduced. Simultaneously processed (floating in same vessel) test filters, bearing glutaraldehyde-conjugates of brain protein with various amino acids, showed no changes in staining intensity or specificity after trypsin. The result suggest that the ability of the Glu antigens to react with antibody is restricted in tissue heavily crosslinked by glutaraldehyde (cf. Brandtzaeg, in Techniques in Immunocytochemistry, Vol.1: 1-75, 1982), particularly in some putative glutamatergic nerve endings. The partial enzymatic cleavage of polypeptide chains may make the antigenic eptiopes more mobile thus promoting interaction with antibody. However, as proteolysis also led to loss of Glu-LI from some sites, this approach is not generally applicable.

In the postembedding immunogold procedure no antigenic epitopes can react except those exposed at the cut surface of the ultrathin sections. This should minimize differencies in antigen accessibility. By this method we have previously found that Glu-LI is preferentially localized in likely glutamatergic neurons in the cerebellar cortex (Somogyi et al., Neuroscience, 19: 1045-1050, 1986). Hippocampi of animals perfusion fixed as above were osmicated and embedded in Durcupan. Ultrathin sections were treated with periodate followed by Glu antiserum no. 13 (1:500 to 1:1000) and colloidal gold particles coated with sheep anti-rabbit Ig. The net particle density over nerve endings bearing the morphological characteristics of the CA3 derived excitatory input and of the mossy fibre boutons had grain densities well in excess of that over other tissue components in CA1 or CA3. Sections of embedded test conjugates that were processed in the same drops of fluids showed that the Glu conjugate had a grain density at least 100 x that of other amino acid conjugates or background. The results suggest that Glu indeed is concentrated in putative glutamatergic nerve endings in vivo. - Supported by NAVF.

434.2 DISTRIBUTION OF GLUTAMATE- AND ASPARATE- LIKE IMMUNOREACTIVITY IN THE RAT CENTRAL NERVOUS SYSTEM.
C. Toomim, P. Petrusz* and K. McCarthy* (Departments of Anatomy and Pharmacology, University of North Carolina, Chapel Hill, NC 27514).

Serial sections of rat brain and cervical spinal cord were stained with antisera prepared against aspartate (Asp) and glutamate (Glu) conjugated with glutaraldehyde to hemocyanin. Female rats were perfused with 1% paraformaldehyde in phosphate buffered saline (PBS) followed by 4% paraformaldehyde-0.5% glutaraldehyde in PBS. The tissues were cut on a vibratome at 30-40µm. The sections were defatted in 30%-75% ethanol before incubation in either Anti-Asp (1:5000) or Anti-Glu (1:7500), and processed for peroxidase immunocytochemistry.

When these procedures are followed, the following statements concerning perikaryal staining are generally true: All nuclei which stain with Anti-Asp, also stain with Anti-Glu. Staining appears to be only in neurons, not glia or ependyma. A greater percentage of neurons in the caudal portions of the CNS stain than in the rostral areas, and staining is usually more intense in the caudal areas. Staining with Anti-Glu was generally less pronounced than that with Anti-Asp. The areas, which contain intensely labelled perikarya include: olfactory bulb (mitral and basket cells), forebrain (zona incerta, subthalamus, habenula, cingulate cortex, diagonal band, islands of Calleja, septum), midbrain (substantia nigra, red nucleus, oculomotor nucleus, lateral lemniscus, interpeduncular nucleus), pons-cerebellum (deep cerebellar nuclei, vestibular nuclei, cochlear nuclei, raphe nuclei, some parts of the reticular formation, spinal, principal and motor trigeminal nuclei, facial nucleus, superior olive, trapezoid body), medulla (dorsal column nuclei, spinal trigeminal nucleus, reticular formation, raphe nuclei, and inferior olive, hypoglossal nucleus, linear nucleus and nucleus ambiguus), cervical spinal cord (all layers).

In addition, many other areas, such as the thalamus, superior and inferior colliculi, and cortex contain less intensely or variably stained perikarya. Other areas, such as the hypothalamic magnocellular nuclei stained in some animals, but not all.

Staining in paraffin-embedded, 5µm thick, trypsin-treated sections usually mimicked that found in vibratome sections.

This work was supported by NIH grant NS21696.

434.3 GLUTAMATE-LIKE IMMUNOREACTIVITY REVEALED BY MONOCLONAL ANTIBODY AND SENSITIVE STAINING METHOD.
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Although Glu is supposed to be a major excitatory transmitter, relatively little is known about its localization. A method allowing to visualize Glu-like immunoreactivity (Glu-LI) at the light microscopic level by means of a monoclonal antibody (mAb) is described. - Mice were immunized with a glutaraldehyde-linked Glu-BSA conjugate, and hybridomas were screened by ELISA for production of antibodies recognizing Glu- but not Asp-BSA. Immunoreactivity of mAb 2D7 as determined by antibody dilution tests in ELISA was more than 1000 times weaker for glutamine-, taurine- and even weaker for other amino-acid- and dipeptide-BSA conjugates than for Glu-BSA. Reactivity with Glu-BSA as measured in ELISA or determined by immunocytochemistry was abolished following preincubation of mAb 2D7 with Glu- BSA at micromolar concentration, whereas an about 1000 times higher concentration was needed with homocysteic acid- and even higher ones with the other amino-acid-(glutamine-, taurine-, etc.) and dipeptide-BSA conjugates tested, and preincubation with free Glu was ineffective. - Semithin sections from glutaraldehyde-fixed, plastic-embedded rat brain tissue were etched and stained by a combination of the indirect unlabeled peroxidase-antiperoxidase (PAP) method and silver enhancement of the diaminobenzidine reaction product. Only this amongst several other immunohistochemical methods tried produced labeling patterns which showed terminal-like elements in brain regions such as hippocampus and cerebellum and which were mostly consistent with already available information on systems using Glu as neurotransmitter. Particularly striking was the staining of elements reminiscent of hippocampal mossy fiber terminals and endings of the cerebellar granule cells.

434.4 GLUTAMATE IMMUNOREACTIVITY IN CORTICO-CORTICAL AND CORTICOFUGAL NEURONS OF RATS.
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Various experimental evidence supports a neurotransmitter role for glutamate in cortical neurons. This has been further demonstrated using an antiserum, raised in rabbits, against glutamate conjugated to hemocyanin with glutaraldehyde (Hepler et al., 1987). By this immunocytochemical method it has been shown in rats and monkeys that glutamate positive cortical neurons are primarily located in layers III,V and VI (Conti et al., 1987). Since these layers also contain the bulk of projecting cortical neurons, it is likely that these neurons use glutamate as neurotransmitter. It remains to be established whether all or only a fraction of projecting neurons are glutamate-positive. This question has been approached by a double-labeling technique: peroxidase immunocytochemistry to visualize glutamate, and retrograde transport of the fluorescent dyes, diamidino yellow (DY) or fluorogold (FG). In rats, either DY (2% in D.W.) or a mixture of DY and FG (1% and 2%, respectively) was injected in the cervical spinal cord, or in the dorsal column nuclei, or in the ventral nuclei of the thalamus, or in the motor cortex (MI). After a survival time of 8-10 days the animals were perfused with carbodiimide or 4% paraformaldehyde followed, in either case, by postfixation in 4% paraformaldehyde. The brains were cut in 15µm serial frontal sections and processed for immunocytochemistry. In all cases not every cortical neuron labeled by the fluorescent dye was also glutamate-positive. The percentage of double-labeled to fluorescence-labeled neurons varied in different layers of the same cortical area. In rats with injection in motor cortex, double labeled cells were mainly in layers II,III and V of ipsilateral primary somatosensory cortex (SI;50% in II, 65% in III, 70% in V) and secondary somatosensory cortex (SII;40%,46%, 57%), and in contralateral MI (35%,60%,62%). After thalamic injections, double-labeled cells in the ipsilateral layer VI were between 55% and 60% in both SI and MI. After injection of the dorsal column nuclei, double-labeled cells in the contralateral layer V were 76% in MI, 73% in SI, and 56% in SII. Double-labeled neurons after spinal cord injections were between 65% and 75% in the contralateral layer V of MI and SI, and 50% in SII. The results suggest that glutamate is the main though not the only neurotransmitter in cortical projecting neurons and that the ratio of glutamergic to non-glutamergic neurons may vary for different cortical layers and different projection pathways.
Supported by USPHS grant NS 16264

434.5 GLUTAMATE AND SUBSTANCE P CO-EXIST IN THE SAME PRIMARY AFFERENT TERMNAL IN THE SUPERFICIAL LAMINAE OF THE RAT SPINAL DORSAL HORN. S. DeBiasi*, S.L. Van Eyck, P. Petrusz* and A. Rustioni (SPON.: S.P.Schneider). Depts. of Anatomy and Physiology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

A role for glutamate as neurotransmitter in at least some dorsal root ganglion (DRG) neurons has been proposed on the basis of mainly pharmacological and electrophysiological evidence. Sixty to 70% of rat DRG neurons are labeled with an antibody prepared against glutamate (Glu) conjugated to hemocyanin with glutaraldehyde; Glu-positive neurons also contain substance P (SP) (Battaglia et al., 1987). Co-existence of Glu and SP in the same DRG perikaryon, however, does not prove that these substances coexist and are co-released at the same synaptic terminal. For instance, immunoreactivity for Glu in DRG somata could be related to metabolic functions rather than neurotransmission. Experiments were therefore performed to explore more directly the possible co-release of these two substances by means of EM post-embedding double-labeling of terminals in the spinal dorsal horn, using antibodies against Glu and SP.

Rats were perfused with 4% paraformaldehyde and 0.5 to 1% glutaraldehyde. Vibratome sections of the spinal cord were embedded in Epon with or without post-fixation in 0.5-1.0% osmium tetroxide. Thin sections from samples of the superficial laminae of the dorsal horn were incubated in the first primary antiserum and then in species-specific IgG adsorbed to 10 nm gold particles. After the first cycle of immunostaining, free anti-IgG binding sites were denaturated by hot paraformaldehyde vapors and the grids were then incubated in the second primary antiserum and in a gold probe of 20 nm. Since both antisera were raised in rabbits, after the first cycle of immunostaining, free anti-IgG binding sites were denaturated by hot formaldehyde vapors.

Both single- and double-labeled terminals were found. SP labeling is present over large granular vesicles in dome-shaped boutons contacting a single dendrite in the plane of the section, and in scalloped endings, typical of primary afferent fibers, contacting several profiles, some of which contain vesicles. Glu-labeling is present over large, clear vesicles and in the axoplasm of scalloped terminals. About 10 to 15% of the scalloped terminals are labeled by both antisera. The demonstration of co-existence of Glu and SP gives further support to the hypothesis that the initial burst of monosynaptic excitatory potential followed by a prolonged depolarization in dorsal horn neurons activated by repetitive dorsal stimulation (Urban and Randic, 1984) results from co-release of Glu and SP from the same DRG terminal.

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434.6 N-ACETYLASPARTYLGLUTAMATE DISTRIBUTION IN THE RODENT SPINAL CORD IN SITU AND IN DISSOCIATED CELL CULTURE. M. Cassidy, J. R. Moffett, M. A. A. Namboodiri, C. B. Cangro, E. A. Neale and J. H. Neale. Dept. of Biology, Georgetown University, Wash. D. C. 20057 and Lab. Developmental Neurobiology, NICHD, NIH, Bethesda, MD.

The nervous system dipeptide, N-acetylaspartylglutamate (NAAG), has been detected in very high concentrations in the spinal cord. As reported previously by others, NAAG-immunoreactivity (IR) was concentrated within a variety of neuronal perikarya throughout the gray matter. Consistent with this, NAAG-IR was detected in some, but not all, myelinated ventral root axons cut in cross section. Additional myelinated axons expressing NAAG-IR were abundant in the ventral and lateral funiculi. Analysis of these ascending and descending tracts following spinal hemisection is in progress. Intense NAAG-IR was present in the dorsal horn of the spinal cord, particularly in the most superficial layers. Some large, irregular IR neurites also were found in this region, suggesting a possible dendritic distribution of the peptide in dorsal horn.

We previously reported the presence of NAAG-IR within the large primary spinal sensory neurons. In an attempt to determine the contribution of primary sensory afferents to the dorsal horn IR, tissue was examined following unilateral dorsal rhizotomy. This produced little detectable difference in the intensity of dorsal horn staining, but eliminated the IR signal normally found in myelinated dorsal root axons. The rhizotomy also eliminated IR in the ipsilateral direct dorsal column pathway of the dorsal funiculus, which contained clearly labeled myelinated axons on the contralateral side and in control spinal cords. We interpret this to mean that spinal sensory neurons convey NAAG in their ascending collateral axon branches. In contrast, most other axons in the dorsal funiculus were unlabeled.

Substantial perikaryal NAAG-IR was observed in murine spinal cord cells which had developed for several weeks in dissociated culture. Both spinal cord and spinal sensory neurons expressed the peptide IR. These cell cultures will be used to study the biosynthesis of NAAG and to evaluate the possibility of its release upon neuronal depolarization. (Supported by NIDA grant DA 02297)

434.7 ULTRASTRUCTURAL LOCALIZATION USING MONOCLONAL ANTIBODIES OF GLUTAMATE DEHYDROGENASE IN RAT BRAIN. J.E. Madl*, J.R. Clements, A.J. Beitz, R.J. Wenthold and A.A. Larson. Dept. of Vet. Biology, U. of Minnesota, St. Paul, MN 55108 and NIH, Bethesda, MD.

Glutamate dehydrogenase (GDH) may play a major role in the metabolism of glutamate in the vertebrate CNS. Glutamate and its analogs are neurotoxic and studies have suggested that deficiencies of GDH may be found in some individuals with olivopontocerebellar atrophy. Monoclonal antibodies to bovine liver GDH were produced and found to be immunoreactive for rat brain GDH on immunoblots and ELISA. One monoclonal antibody, GD-2, reacted with GDH treated with either paraformaldehyde or glutaraldehyde, the fixatives that were used for tissue fixation in these studies. Immunocytochemical staining of rat cerebellum with GD-2 revealed intense staining of small, punctate structures identified as mitochondria following electron microscopic examination. Intensely labeled mitochondria were observed primarily in Bergman's glia in the cerebellum. Rarely, labeling of mitochondria in neuronal profiles, predominantly dendrites, was observed in the cerebellar cortex. Large numbers of unlabeled mitochondria were observed in many cell types throughout the cerebellum. Intensely labeled mitochondria were observed in neurons and glia of other regions of the CNS including the hippocampus. Immunocytochemical staining was inhibited by preincubation of the antibody with GDH. The intense immunolabeling of a subpopulation of mitochondria found in only a fraction of the glia and neurons present in the CNS suggests that GDH may be present in high concentration in this subset of mitochondria. This compartmentalization of GDH in a subset of mitochondria may prove to be important in understanding the pathophysiologic mechanisms associated with a variety of excitotoxin-related disease processes. Supported by USPHS grants NS01105, NS07813, DA04090, DE06682 & NS19208.

434.8 GLIAL UPTAKE OF GLUTAMATE: AN IMMUNOHISTOCHEMCICAL STUDY IN THE HIPPOCAMPAL SLICE K.J. Anderson, R.J. Bridges, R. J. Wenthold, and C.W. Cotman, Department of Psychobiology, Univ. California, Irvine, CA 92717 and NIH, Washington, D.C. 20205.

Studies on the excitatory amino acid glutamate have suggested that this transmiter is inactivated by rapid and specific uptake into glia where it is converted to glutamine. The use of anitbodies directed against amino acids has made it possible to study aspects of the "glutamate cycle" using immunohistochemical methods. In particular, studies by Ottersen and Storm-Mathisen have shown evoked "release" of glutamte by neurons and subsequent "uptake" by astrocytes, in the hipppocampal slice preparation. We have extended these studies to include the detailed pharmacological characterization of glutamate release by neurons and the subsequent uptake by glia. Slices of rat or guinea pig hippocampi (450 μm) were placed into ice-cold Krebs solution and continuously bubbled with 95% O_2/5% CO_2. The slices were then placed in an experimental incubation medium at 30°C for 1 hour; after which the slices were rinsed, fixed with aldehydes and re-sectioned at 40 μm. The sections were stained using an affinity-purified antiserum directed against conjugated glutamate. In sections incubated in control buffer for 60 min, dense glutamate-like immunoreactivity (Glu-Li) was seen in the stratum radiatum, oriens and the inner molecular layer of the dentate gyrus. Intermediate staining was seen in the outer molecular layer and stratum lucidum while very little staining was observed in the middle molecular layer and the stratum lacunosum-moleculare. In sections incubated in 40-55 mM [K^+], there was a loss of laminar Glu-Li and an appearance of intensely stained cells which resembled astrocytes. Double labelling with a monoclonal GFAP antibody and glutamate antiserum confirmed the glial identity of these cells. The "release" of Glu-Li from the slices did not occur in the absence of Ca^{2+}. In slices that were exposed to high [K^+] for 60 minutes and then placed into control buffer with the addition of glutamine (0.5 mM) for an additional 30 min, the "normal" pattern of Glu-Li staining was retained. The [K^+]-induced appearance of Glu-Li in astrocytes was blocked by 1 mM D-aspartate, D,L threo-ß-hydroxyaspartate, cysteic acid and SITS. The staining of glia was not affected by 100 μM D-aspartate or D-L APB (200 μM). The addition of exogenous glutamate (50-500 μM) to control buffer induced astrocyte staining only in the absence of TTX (0.5 μM), indicating that this staining was a result of glutamate stimulation of glutamatergic neurons. Similar results were seen with the addition of 100 μM NMDA. Depolarization of the slices by veratridine (100 μM) also resulted in the depletion of the normal Glu-Li staining pattern, however, no astrocyte staining was observed, indicating that Na^+ channels are required for uptake or retention of Glu-Li by astrocytes. Using this novel approach to study glutamate uptake and release, we have provided data to support the idea that transmitter pools of glutamte are "cycled" through astrocytes and that the transport of glutamate is regulated by a specific uptake site(s) which is amenable to pharmacological manipulations.

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434.9 EFFECTS OF KAINATE AND N-METHYL-D-ASPARTATE ON THE RELEASE OF TRITIUM FROM RAT RETINA FRAGMENTS PRELABELLED WITH ^{3}H-CHOLINE. L. L. TRUEX AND M. M. FOREMAN. Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN, 46285.

Glutamate and aspartate have been proposed to be transmitters of bipolar cells within the vertebrate retina (Ikeda and Sheardown, Vision Res. 23:1161-1174, 1983). These transmitters have been found to modulate the in vivo release of acetylcholine (ACh) from amacrine cells through the activation of the kainic acid (KA), quisqualic acid (QA) and N-methyl-D-aspartic acid (NMDA) receptor subtypes (Cunningham and Neal, J. Physiol. 366:47-62, 1985). The focus of the present studies was to evaluate the in vitro effects of KA and NMDA using the efflux of tritium from superfused retinal fragments that were prelabelled with ^{3}H-choline as a marker for ACh release. Adult male rats of the Sprague-Dawley strain were decapitated and the eyeballs were removed. The anterior portion of the eyecup and the vitreous humor were removed. The retinal tissue was detached from the eyecup by irrigation with buffer containing 118 mM NaCl, 4.2 mM KCl, 25 mM $NaHCO_3$, 1.3 mM $CaCl_2$, 1.2 mM $MgCl_2$ and 11.1 mM beta-D-glucose which was saturated with 95/5 % O_2/CO_2. Retinal tissue was removed by bisection of the optic nerve, placed in buffer and dissected into fragments of approximately 1/4 th of the retinal mass. These fragments were incubated for 30 min at 25^0 C in buffer containing 100 nM ^{3}H-choline. Following this prelabelling procedure, individual fragments were placed in 200 ul tissue chambers and superfused with buffer at a rate of 400 ul/min. After a 45 min equilibration period, the fragments were exposed to buffers containing various concentrations of KA or NMDA. A 5 min exposure to 0, 1, 10 or 100 uM KA resulted in increases of the relative fractional release (RFR) of 0.18 ± 0.06, 0.34 ± 0.07, 1.39 ± 0.27 and 3.09 ± 0.70 percent, respectively. The KA evoked release of tritium from these fragments was attenuated by the presence of gamma-D-glutamylaminomethylsulfonate (GAMS), a KA receptor antagonist, but not by D/L-2-amino-7-phosphonoheptanoate (D/L-AP-7), a selective NMDA antagonist. In the same buffer, 100 uM NMDA evoked a RFR of only 0.36 ± 0.08 percent, whereas in Mg^{2+}-free buffer NMDA at the same concentration evoked a RFR of 1.39 ± 0.20 percent. NMDA in concentrations of 0, 1, 10 and 100 uM evoked RFR's of 0.03 ± 0.09, 0.17 ± 0.05, 0.75 ± 0.44 and 1.33 ± 0.26 percent, respectively. The tritium release evoked by NMDA was abolished when D/L-AP-7 was added to the superfusion buffers, but was unaffected by the presence of GAMS. The in vitro release of tritium from ^{3}H-choline prelabelled, rat retinal fragments appears to resemble the previously described in vivo release of ACh. Thus, the evoked release of tritium from ^{3}H-choline prelabelled, retinal tissue appears to be a simple method to obtain responses with a relatively high sensitivity to KA and moderate sensitivity to NMDA.

434.10 QUANTITATION OF N-ACETYL-ASPARTYL-GLUTAMATE (NAAG) IN TISSUE BY A COMPETITIVE LIQUID PHASE RADIOIMMUNOASSAY. A.S. Guarda*, L. Ory-Lavollée, M.B. Robinson, R.D. Blakely, G. Forloni, and J.T. Coyle. (SPON: D. Braitman) Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

NAAG is an acidic neuropeptide which has been proposed to play a role in excitatory synaptic transmission within the rodent CNS. Regional variations in NAAG levels across the rat CNS have been documented by HPLC techniques. Sensitivity constraints imposed by this method however, prevent the detection of NAAG in quantities below the nmol level. Accordingly, we have developed a liquid phase radioimmunoassay (RIA) capable of detecting pmol quantities of NAAG.

Methanol extracts of rat CNS tissue samples were lyophilized, and NAAG was quantitated by HPLC (Blakely et al., J. Neurochem. 47 (1986) 1013-1019) and by liquid phase radioimmunoassay. The RIA was performed with NAAG as the standard and [^{3}H]-NAAG (52.4Ci/mmol) as the displaceable radioligand. Tissue extracts, in amounts yielding between 40-60 % displacement, were incubated with ammonium sulfate precipitated immune serum (RBLOLγ) and [^{3}H]-NAAG (total volume of 200 µl). After 48 hours, the assay was terminated by precipitation with a goat anti-rabbit gamma globulin, followed by filtration.

NAAG displacement followed a sigmoidal curve with an IC_{50} of 4.5-6.5 pmol per tube. The sensitivity of this assay was 1 pmol per tube. N-acetyl-aspartic acid (NAA) and aspartyl-glutamate displacement curves paralleled the curve obtained for NAAG with IC_{50}'s of 350 pmol and 75 nmol, respectively. Glutamate and aspartate displaced less than 10 % at 500 nmol. Thus, this assay was highly specific for NAAG with respect to two major brain contaminants, glutamate and aspartate, and showed 50-fold specificity for NAAG with respect to NAA.

Comparison of regional NAAG levels determined by HPLC and by RIA revealed a high correlation (r = 0.99). Levels of NAAG-like immunoreactivity determined by RIA were approximately 3 nmol/mg protein higher than those measured by HPLC. RIA analysis of HPLC fractions from several regions revealed only two peaks of immunoreactivity, co-chromatographing with NAA and NAAG. Correction of RIA displacement values by subtraction of endogenous NAA crossreactivity yields essentially identical levels for NAAG obtained by HPLC and by RIA across eight brain regions (r = 0.98, slope =1.1).

We have performed radioimmunoassays of microdissected tissue from various brain nuclei including the Raphe and red nuclei, the locus ceruleus, the dorsal and ventral horns of the spinal cord, striatum, and cortex. These data show a correlation between NAAG-like immunostaining in carbodiimide perfused tissue with a different antiserum (KJK1), and NAAG-like immunoreactivity quantitated by RIA. The 1000-fold greater sensitivity afforded by this RIA over HPLC indicates that this assay will be useful for quantitating NAAG in µg amounts of brain tissue, in brain perfusates, and in peripheral tissues with low amounts of NAAG. [Supported by USPHS # NS 13584 and a postdoctoral fellowship (# 07870) to MBR]

434.11 EXITATORY AMINO ACID STIMULATED RELEASE OF [^{3}H]-GAMMA-AMINO-BUTYRIC-ACID FROM HIPPOCAMPAL NEURONS IN VITRO K.M. Harris* and R.J.Miller (SPON: R. McCrea) Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637

Various amino acids have been suggested to be excitatory neurotransmitters in the mammalian central nervous system (CNS). The mechanism of action of these amino acid neurotransmitters has been extensively studied in the hippocampus. One population of interneurons in the hippocampus utilizes gamma-amino-butyric acid (GABA) as a neurotransmitter. In the present study, we have investigated the release of GABA from hippocampal neurons and its regulation by excitatory amino acids. Neurons dissected from fetal (embryonic day 18) rat hippocampus were dissociated, grown in primary culture and used experimentally after 8-10 days in vitro. Immunohistochemical studies showed that GABA and glutamate containing neurons are present in significant numbers in these cultures. Cultures of hippocampal neurons accumulated and stored [^{3}H]-GABA and [^{3}H]-glutamate. Preaccumulated [^{3}H]-GABA was released when the cells were depolarized with either 50 mM K^+ or 30 µM veratridine, however [^{3}H]-glutamate release was not detected. [^{3}H]-GABA release was blocked by 100 µM Cd^{2+}. The dihydropyridines, BAY K 8644 and nitrendipine, 1µM, had no effect on 50 mM K^+ stimulated release. 3 µM tetrodotoxin (TTX) blocked release stimulated by 30 µM veratridine but had no effect on 50 mM K^+ stimulated release. Veratridine stimulated release was 4-fold higher than 50 mM K^+ stimulated release. [^{3}H]-GABA release was also stimulated by 100 µM glutamate and by 100 µM N-methyl-D-aspartate (NMDA). Glutamate stimulated release was slightly greater than release stimulated by NMDA. NMDA stimulated release was completely blocked by 100 µM Cd^{2+}, while glutamate stimulated release was only partially blocked. Neither the dihydropyridines, BAY K 8644 and nitrendipine (1 µM) or TTX (3 µM) had any effects on the excitatory amino acid stimulated release. NMDA stimulated release could be blocked by 1mM Mg^{2+} and a competitive NMDA receptor antagonist, 3-((±)-2-carboxy-piperazin-4-yl)-propyl-1-phosphonic acid (CPP), 100 µM. Changes in $[Ca^{2+}]_i$ were monitored in single hippocampal neurons with the fura-2 based microspectrofluorometric technique. Basal $[Ca^{2+}]_i$ in the cultured hippocampal neurons averaged 100 nM. $[Ca^{2+}]_i$ increased to 234 nM and 291nM when the cells were stimulated with 50 mM K^+ and 30 µM veratridine, respectively. The veratridine response was blocked by 3 µM TTX. NMDA and glutamate caused dose dependent increases in $[Ca^{2+}]_i$, EC_{50} =3 µM and EC_{50} =10 µM, respectively. Maximally effective concentrations of NMDA (100 µM) and glutamate (100 µM) raised $[Ca^{2+}]_i$ to 241nM and 315 nM, respectively. The response to NMDA (10 µM) was also enhanced by 30 µM glycine. Thus GABA can be released from cultured hippocampal neurons by raising $[K^+]_o$ and by excitatory amino acids. Ca^{2+} influx via dihydropyridine insensitive Ca^{2+} channels appear to play a role in both responses.

434.12 POSSIBLE CONTAMINATION OF QUISQUALIC ACID BY NATURALLY OCCURRING GLUTAMIC ACID DETECTED BY QUANTITATIVE AUTORADIOGRAPHIC BINDING ASSAY AND HPLC ANALYSIS. J. J. Cha, Z. Hollingsworth*, J. T. Greenamyre, J. Collins*, J. B. Penney, and A. B. Young. Neuroscience Program and Department of Neurology, University of Michigan, Ann Arbor, MI 48104 and Department of Chemistry, City of London Polytechnic, London EC3N ZEY, U. K.

Quisqualic acid (QA), an analog of glutamic acid (Glu), preferentially excites a subclass of glutamate receptors with high (nanomolar) affinity. Commercially available QA is purified from the berries of *Quisqualis fructus* and *Q. indica*. The isolation procedure employed is such that a small but significant amount of contaminant Glu could remain in a sample of commercial QA. The presence of contaminating Glu could produce spurious results in experiments in which QA is tested. We present evidence of the presence of Glu in commercially available QA. Further, the amount of contamination varied among different batches of QA tested.

Different batches of QA were tested for their ability to displace [^{3}H]glutamate binding in sections of rat brain, using a quantitave autoradiographic binding assay. Brain sections were incubated with [^{3}H]glutamate (200nM) in the presence of varying concentrations of QA. QA displaced [^{3}H]glutamate in a biphasic manner, corresponding to two binding sites for QA. The K_i value for the "high affinity" site (14 to 30nM) was not significantly different among batches of QA tested. In contrast, the K_i value for the "low affinity" site (25 to 100µM) was significantly different for different batches. QA which was chemically synthesized rather than purified from a natural source also exhibited a biphasic displacement of [^{3}H]glutamate binding, with a K_i value for the high affinity site which was not significantly different from commercial batches. The K_i value for the low affinity site (250µM), however, was much greater than for commercial batches.

Reverse-phase HPLC analysis revealed the presence of several small contaminant peaks in all batches of QA tested. One peak was found to co-elute with exogenously added Glu. In commercial batches of QA the size of this peak was greater than in synthetic QA.

These data are consistent with the possibility that commercial QA may be contaminated with small amounts of Glu. The "low affinity" QA binding sites may represent non-quisqualate (*i.e.* NMDA) receptors. At high concentrations of QA, contaminant Glu could interact with these non-QA receptors. Pure QA may therefore be much more of a selective agonist at QA receptors than has been previously believed.

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434.13 CHARACTERIZATION OF POTENTIAL NEUROACTIVE SUBSTANCES RELEASED FROM RAT BRAIN IN VITRO. H.J. Keller*, K.Q. Do*, M. Zollinger*, K.H. Winterhalter* and M. Cuénod. Brain Research Institute, Univ. Zürich, CH-8029 Zürich, Switzerland.

As no established neurotransmitters have been ascribed to some major CNS pathways, a screening of neuroactive substances has been performed by selecting compounds which are released from rat brain slices upon K^+-depolarization in a Ca^{++}-dependent manner (Do, K.Q., Mattenberger, M., Streit, P., and Cuénod, M., J. Neurochem., 46: 779, 1986). 9-Fluorenylmethylchloroformate (FMOC-Cl) pre-column derivatization was chosen to analyse the perfusates because of several advantages: (1) The derivatization proceeds under mild conditions in aqueous solution and is complete in 30 sec. (2) The derivatives are stable at 4°C for weeks. (3) The incorporation of the fluorescent FMOC-group allows high sensitivity of detection as well as good separation of primary and secondary amino acids with reversed-phase HPLC. (4) FMOC-derivatives can be deprotected under mild basic conditions, thus the original compound can be recovered in high yield and can be used for further analytical purposes. Compounds which showed a Ca^{++}-dependent release were collected from successive HPLC runs, purified at a different pH of the mobile phase and structurally elucidated by amino acid analysis and fast atom bombardment mass spectrometry. One of these compounds was identified as 9-fluorenylmethoxycarbonylpyroglutamate, a to date unknown side-product of FMOC-derivatization of glutamate. Furthermore a group of substances termed P25c,d,e,f could be isolated in the presence of the protease inhibitor iodoacetamide. P25c was shown to contain at least Glx and a base labile group. P25f gave after hydrolysis with 6N HCl an unusual polar amino acid as a constituent.

434.14 ELECTROACUPUNCTURE EVOKED RELEASE OF GLUTAMATE IN THE HIPPOCAMPUS MONITORED WITH DIALYSIS-PERFUSION IN VIVO. D.Z. Lee*, J.W. Lazarewicz* and A.Y. Sun. Sinclair Comp. Med. Res. Farm and Biochem. Dept., University of Missouri, Columbia, MO 65203 and Medical Research Center, Polish Academy of Science, Warsaw, Poland.

Electroacupuncture (EA) has been used for the amelioration of pain. The EA analgesia may be similar to analgesia produced by electrode stimulation. We have demonstrated previously that EA stimulation at acupuncture point ST-36 alters catecholamines in different brain regions, especially in hypothalamus and caudate nucleus (Sun et al. 1984). The effect of EA on neural activities may depend also on the frequencies used (Lee & Sun, 1984). We have employed a microdialysis technique to study the glutamate (Glu) release in hippocampus in vivo. We have demonstrated that EA stimulation mediates the release of Glu from hippocampus similar to the release evoked by high K^+ or veratridine. The release is dependent on Ca^{++} and blocked in the presence of EGTA. Low frequencies (2-4 Hz) did not show any Glu release. However, higher frequencies (50-200 Hz) lead to a significant increase in Glu release. Results indicate that higher frequency of EA stimulation may activate other afferent pathways causing unusual behavior and synaptic transmission.

Lee, D.Z. and Sun, A.Y. (1984) Neurochem. Res. 9 669-678.
Sun, A.Y., Boney, F. and Lee, D.Z. (1984) Neurochem. Res. 10 251-258.

434.15 A SIMPLE AND RAPID METHOD FOR MEASURING ASPARTATE AMINOTRANSFERASE ACTIVITY IN SPECIFIC REGIONS IN THE NERVOUS SYSTEM. D. Garrison*, J. Beattie* and M.A.A. Namboodiri. Dept. of Biology, Georgetown University, Washington, D. C. 20057.

Aspartate aminotransferase (EC 2.6.1.1., AAT) catalyses the transamination of aspartate in the presence of α-keto glutarate to form oxaloacetate and glutamate. Based on immunohistochemical localization data, it has been proposed that AAT is a marker enzyme for aspartate/glutamate transmitter pathways in the nervous system. However, only limited progress has been made in understanding the possible role of AAT in the regulation of the synthesis of the neurotransmitter pool of aspartate/glutamate. This is due, in part, to the lack of a simple and sensitive method for measuring changes in the activity of AAT in specific areas of the nervous system under different physiological and pharmacological conditions. We report here an assay procedure which has been developed recently in our laboratory.

To assay AAT activity, a 10 ul aliquot of potassium phosphate buffer (0.1 M, pH 7.4) containing L-[2,3-^{3}H] - aspartate (SA=5 μCi/μ mole, 20 mM) and α-keto glutarate (20 mM) is incubated (30 min. 37 C) with a 10 ul aliquot of rat brain homogenate prepared in the phosphate buffer containing NP-40 (0.15%). Following incubation, the reaction is terminated by adding HCl(0.1N, 180 ul). As a result of the transamination of aspartate, which results in the formation of oxaloacetate, the tritium atoms at positions 2 and 3 of aspartate are released into the aqueous medium. The released tritium is separated from the radiolabelled aspartate by passing the reaction mixture over a cation exchange column (Bio-Rad AG 50 W-X8, 7 x 30 mm) equilibrated with HCl (0.05N). The column is washed with 2 ml HCl and the radioactivity in the combined flow through and wash is determined. Using anion exchange HPLC followed by evaporation, we determined that this radioactivity is associated with water. Also, we have found that when ^{14}C-aspartate, instead of 2,3 ^{3}H-aspartate, is used in the assay, the radioactivity in the product co-migrates with oxaloacetate in anion exchange HPLC. The product formation, as determined by the amount of tritiated water formed, was found to be linear with time up to 120 min and with tissue concentration in the 0.05 to 100 μg range. Using an extensively dialysed brain homogenate preparation, we have found that product formation is dependent on the concentrations of both aspartate and α-keto glutarate. Using this assay procedure it should be possible to accurately measure AAT activity in specific cell zones and projection pathways in the nervous system. Thus the development of this simple and sensitive assay procedure is expected to stimulate study the role of AAT in the synthesis of aspartate/glutamate in putative acidergic neuronal pathways. Research supported by NIDA Grant DA 02297 to J. H. Neale.

434.16 HPLC-EC ANALYSIS OF PRIMARY AMINO ACIDS IN SALINE SOLUTIONS. V.R. Roettger* and M.D. Goldfinger. Department of Physiology and Biophysics, Wright State University, Dayton, Ohio 45401-0927.

This work examines parameters of the separation and amperometric detection of eight primary amino acids: Aspartate[ASP], Glutamate [GLU], Glutamine[GLN], Homoserine[HS], Glycine[GLY], Taurine[TAU], Alanine[ALA], and GABA. Isocratic mobile phase [0.1M Na-Phosphate-2H buffer-pH 5.5-plus MeOH, see below] is filtered, degassed, and pumped [BAS PM-3OA] at 1.6 ml/min through a C18 column [5 um reverse-phase, 4.6 X 250. mm, T=34°C., P≅3K psi] to an amperometric detector [BAS LC-4B, V=+0.7V] with output displayed on a chart recorder and digitized [2 or 4/sec; 8 bits] for disk storage and replotting.

Amino acids are detected as isoindole derivatives formed in a precolumn reaction with o-phthalaldehyde[OPA,Pierce] and B-mercapto-EtOH[BME,Pierce]. The derivatizing solution [DS] consisted of 9 ml 0.1M Na-tetraborate[pH 9.1] plus 1 ml OPA[27 mg/ml EtOH]. Excess BME content in the DS was maintained with 1 ul aliquots of BME[1 ul + 100 ul borate]. DS BME content was monitored amperometrically between runs. An excess BME peak height was established [=11.3 nA] which provided maximal detection of 100 picomoles of the 8 amino acid derivatives together or singly. The controlled DS BME content was at least twice the amount below which detectability declined, and provided a short-duration BME peak to minimize interference with detecting rapidly-eluting derivatives [ASP;GLU;GLN]. All amino acid solutions were made in artificial cerebrospinal fluid[pH 7.4] (1).

Parameters of the derivatizing reaction affected derivative detection. Decreasing the reaction time from 2.5 to 1.5 min enhanced GLY,TAU,and GABA peaks[13,28,67 %, resp.]. Decreasing the DS:sample volume ratio from 100 ul:25 ul to 25 ul:25 ul increased each peak height between 95%[ALA] to 367%[GABA].

Mobile phase composition affected detection sensitivity and precision. Two mobile phases were tested, differing in Buffer:MeOH volume ratio; MP1,70:30 and MP2,60:40. Derivatizing conditions were 1.5 min reaction time and 25 ul DS:25 ul sample. Sensitivity was the slope of the concentration curve(2) obtained between 1-100 pmoles. In both MPs, sensitivity was inversely related to elution time, due to derivative instability(3). Sensitivities[pAmp/pmole] ranged from 109[GLU] to 15[GABA] in MP1, and from 238[ASP] to 35[GABA] in MP2. Total elution time was 29 min in MP1 and 15 min in MP2. Detection precision in MP1 exceeded that in MP2. Coefficients of variation in MP1 ranged from 2%[TAU] to 4%[HS], and in MP2 from 4%[GLU] to 8%[GLY]. In MP2, background peaks were larger and more numerous, and GLN detection was not quantitative.

The detection limits [in MP1- ASP,GLU,GLN: 0.625 pmole; HS:1.25 p-moles; GLY,TAU,ALA,GABA:2.5 pmoles] may be amplified with no extra baseline noise by solvent elimination[Savant Speed-Vac].
REF.s:(1)M.D.Goldfinger,Life Sci.37:1765,1985;(2)P.Kissinger,Cur.Sep. 7:91,1986;(3)L.Allison et al.Analyt.Chem.56:1089,1984.

435.1 ALTERED RESPONSIVENESS OF CAUDATE NEURONS TO IONTOPHORETICALLY APPLIED NEUROTRANSMITTERS IN AGED CATS. M.S. Levine, C. Cepeda, C.D. Hull and N. A. Buchwald. Mental Retardation Research Center. University of California, Los Angeles. 760 Westwood Plaza. Los Angeles, CA 90024.

Previous studies from our laboratory have demonstrated that decreases in excitation occur in caudate nucleus neurons in aged cats. These changes may be mediated by postsynaptic and/or presynaptic alterations. In order to provide additional information about these changes we assessed the responsiveness of extracellular recorded caudate neurons to iontophoretic application of several putative neurotransmitters that are important in caudate neuronal function. The effects of glutamate (GLU), gamma aminobutyric acid (GABA) and dopamine (DA) were studied in three groups of cats; young adult (1-3 years, n=5), middle aged (6-8 years, n=4) and aged (11-15 years, n=3). Animals were anesthetized with fluothane for the duration of the experiment, paralyzed with flaxedil and artifically respirated with a mixture of nitrous oxide and oxygen. Extracellular recordings were obtained with glass electrodes glued to multibarreled micropipettes from which solutions were iontophoresed. In 1-3 year cats all caudate neurons (31/31) displayed excitatory responses to GLU. In both 6-8 and 11-15 year cats there was about a 15% (42/50) decrease in the proportion of neurons excited by GLU. The proportion of neurons inhibited by GABA remained relatively constant in all age groups (100% (21/21) in 1-3 year animals and 95% (49/50) in aged cats). When DA was applied there was a decrease in the proportion of responsive neurons in both groups of older animals (67% (18/27) in 1-3 year versus 50% (25/50) in both 6-8 and 11-15 year animals). In addition, in both groups of aged cats higher currents were required to produce changes in action potential frequency when DA was iontophoresed. In the aged cats DA also produced increases in the frequency of occurrence of action potentials about 25% of the time. This response rarely occurred in 1-3 year cats. The interaction of the ability of GLU and DA to alter responses evoked by cortical stimulation was also assessed. DA typically inhibited the ability of GLU to augment cortically induced excitatory responses in 1-3 year cats. In both groups of aged cats this type of modulation was not apparent. These experiments provide evidence that the ability of neurotransmitters to alter electrophysiological activity of caudate neurons is affected by the aging process. These alterations begin to occur during middle-aged periods in the cat. Changes in biochemical and pharmacological indices of transmitter function have been observed during these middle-aged periods in other species. Supported by AG-01558 and HD-05958.

435.2 DECREASED EXCITATION IN STRIATAL NEURONS IN AGED RATS REVEALED BY INTRACELLULAR RECORDING. C. Cepeda*, J.P. Walsh, N.A. Buchwald, C.D. Hull, R.S. Fisher and M.S. Levine (SPON: M.K. Shellenberger). Mental Retardation Research Center, UCLA School of Medicine, 760 Westwood Plaza, Los Angeles, CA 90024.

Previous electrophysiological studies from our laboratory have demonstrated reduced excitation in caudate neurons in aged cats. In these extracellular studies there were slower spontaneous firing rates, fewer initially excitatory responses to activation of monsynaptic inputs and elevated thresholds for evoking excitatory responses. In the present experiment intracellular recordings were used to characterize the changes in membrane potential responses underlying the decrease in excitation. Because intracellular recording usually produces less data per experiment, we used rats instead of cats in these studies. Animals were divided into three age groups, young (n=18) 3-5 months, middle-aged (n=8) 12-15 months and aged (n=3) > 24 months. Rats were anesthetized with urethane, paralyzed with flaxedil and artificially respirated. Stimulating electrodes were implanted in frontal cortex and substantia nigra. Intracellular records were obtained from K-acetate-filled micropipettes. Impedances ranged from 30-60 Mohms. To date stable intracellular recordings have been obtained from 60 neurons (30 in young, 20 in middle-aged and 10 in aged rats). Resting membrane potentials of these cells ranged from -40 to -85mV. Action potentials ranged from 35 to 70mV. Differences in the threshold for eliciting both synaptic responses and action potentials occurred between young rats and those in the two older age groups. In all neurons excitatory postsynaptic potentials (EPSPs) could be evoked in response to activation of cortex or nigra but stimulus thresholds were markedly elevated in many neurons in the older animals. In young rats thresholds to evoke EPSPs averaged about 500μA. In the middle-aged group about 15% of the neurons encountered had elevated thresholds (>1mA) to evoke EPSPs while in the aged group over 50% of the neurons had elevated thresholds. Stimulus intensities needed to evoke action potentials were also greatly increased in the older animals. Neurons in both groups of aged rats displayed elevated thresholds for induction of action potentials by injection of depolarizing current pulses through the recording electrode. These findings indicate that reduced excitation in aged animals is due partly to a decrease in the ability of monosynaptic inputs to evoke EPSPs. The reduction appears to be a consequence of a combination of decreased synaptic input and decreased ability of caudate neurons to generate action potentials. These findings also provide a replication of previous data obtained from cats and indicate that reduced excitation may be a major effect of aging in the striatum. Supported by USPHS Grant AG01558 and HD05958.

435.3 BEHAVIORAL EVALUATION OF MOTOR DYSFUNCTIONS IN MUTANT HAN-WISTAR RATS: A MODEL FOR INHERITED DISORDERS OF THE BASAL GANGLIA. L. Hiraide,* J.P. Walsh, C.D. Hull, F.C. Zhou, R.S. Fisher, N.A. Buchwald and M.S. Levine. Mental Retardation Research Center, UCLA School of Medicine, 760 Westwood Plaza, Los Angeles, 90024.

A mutant strain of Han-Wistar rats which carries an autosomal recessive genetic defect is characterized by a progressively developing tremor, ataxia and increased muscle tone in the hindlimbs (Neurosci. Letters 2: 45-49, 1976). A disturbance in gamma aminobutyric acid (GABA) transmission in the basal ganglia has been related to this disorder (Brain Res. 347: 258-267, 1985). This study provides a set of quantifiable indices of the intensity and duration of the progression of these motor symptoms and a baseline from which the effects of treatments designed to ameliorate the disorder can be determined. To date we have quantified behavioral changes in 9 affected rats and 3 normal littermates (homozygote controls). None of the behavioral responses described below are displayed by non-affected littermates. The first detectable sign of the disorder is a decrease in weight gain of affected rats relative to their littermates of the same sex. This begins during the 4th postnatal week. During the 4th and 5th week motor symptoms appear. The first symptom is a rapid tremor of the forepaws which occurs for the duration of the rats' survival. Jaw tremor begins at about 30 days and lasts for the duration of survival. These behaviors become more intense as the disorder progresses. Loss of forelimb support begins at 35-40 days and maximizes at about 60 days. The forelimbs become clenched at about 45 days and remain in that position for the duration of the disorder. At 35-40 days the animals sit with their hindlimbs forward and under their body, display ataxia and locomote by hopping. These behaviors disappear after 60 days as hindlimb support is increasingly lost. Partial loss of hindlimb support occurs at 45-50 days and is complete by 65 days. Hindlimb rigidity occurs at about 60 days of age and becomes more severe as the disorder progresses. Animals have difficulty eating and drinking by 60-70 days and then must be handfed. Animals were sacrificed at about 90 days. These results indicate that the genetic disorder progresses in an orderly fashion, first affecting forelimb and jaw musculature, then forelimb and hindlimb support, locomotion and finally producing rigidity of the hindlimbs. Preliminary observations on 3 of these animals using immunohistochemical staining for glutamic acid decarboxylase, the synthetic enzyme for GABA, revealed decreased terminal staining in the substantia nigra. This genetic defect has the potential to serve as a model for an inherited disorder of the basal ganglia such as Huntington's Disease. Supported by USPHS Grant HD05958.

435.4 THE SOMATODENDRITIC ARCHITECTURE OF CORTICOSTRIATAL NEURONS. R.S. Fisher, M.S. Levine, C.D. Hull and N.A. Buchwald. Mental Retardation Research Center. University of California, Los Angeles. 760 Westwood Plaza. Los Angeles, CA 90024.

Corticostriatal (C) connections have an important physiological role in the basal ganglia by the morphology of their neurons of origin is obscure. We combined the labeling of connectivity (WGA-HRP) and somatodendritic (Golgi-gold toning) markers to rigorously identify the C neurons of young adult cats by correlative light and electron microscopy. We observed both pyramidal and nonpyramidal C neurons in 7 cats. These results are important evidence for the morphological diversity of corticofugal neurons. They also suggest that hetergeneous transmitters associated with each structural type of neuron may mediate a variety of synaptic influences in the C connections.

The C connections included undecussated and decussated axons mainly from rostral neocortex (Areas 4, 6 and prefrontal regions). The principal C neurons were small-to-medium sized pyramidal neurons located in laminae II-VI. These cells had appropriately oriented thick apical dendrites, variable dendritic fields and numerous dendritic spines. The presence of a specialized "apical" dendrite with unsual directions signified the presence of misoriented and inverted pyramidal neurons with C connections in laminae V-VI. The pyramidal neurons received symmetric (major) and asymmetric (minor) axosomatic and axodendritic inputs. The dendritic spines usually received symmetric inputs. Nonpyramidal C neurons were infrequent and located widely in laminae II-VI. These small-to-medium sized neurons had many somatodendtic architectures including star-pyramid, stellate, multipolar and fusiform types. These nonpyramidal C neurons had no apical dendritic specialization even in electron microscopic reconstructions from serial sections. Their dendritic spines were less frequent than those of pyramidal C neurons. Their axosomatic inputs were sparse. All of these types of C neurons were apparent in different neocortical areas and among the decussated and undecussated C pathways. Supported by USPHS HD05958 and AG01558.

435.5 FETAL DEVELOPMENT OF SUBSTANCE P WITHIN BASAL GANGLIA OUTPUT NUCLEI OF THE CAT. M.K. Boylan,*R.S. Fisher, R. Gayek, M.S. Levine, C.D. Hull and N.A. Buchwald. Mental Retardation Research Center, University of California, Los Angeles. 760 Westwood Plaza. Los Angeles, CA 90024.

The initial expression and subsequent fetal development of substance P (SP) within the basal ganglia (BG) of cats occurs before the development of other neurotransmitters (GABA, ACh, and glutamate). These events precede the formation of synapses in the BG. To study the role of SP during development, we analyzed its regional disposition and development in the output nuclei of the BG: globus pallidus (GP), entopeduncular nucleus (EN) and substantia nigra (SN). Light microscopic analysis of substance P-like immunoreactivity was performed in 16 fetal kittens of ages F30 through F60 (gestation period = 65 days). The most important sources of SP within the BG are the neostriatal (NS) medium spiny neurons that provide the principal inputs of the mature GP, EN and SN. SP was seen in the NS as early as F30.

The GP, EN and SN contained sparse SP immunoreactivity at F30 in the form of SP fibers and growth cones. These increased in frequency with fetal aging. Within the GP, SP developed along a caudal-to-rostral and medial-to-lateral gradient. The SP fibers developed in the EN developed from a central core region which extended and became more dense with age. At F30, the SN contained SP-positive fibers and growth cones which emanated from the cerebral peduncle. A dense neurite meshwork of fibers, punctae and growth cones developed rapidly thereafter in all parts of the SN. Interestingly, SP-immunoreactive cell bodies were seen within the fetal SN and GP thoughout development, but they disappeared with advancing age. Such cells have not been observed in the SN or GP of adult cats. They may express SP transiently during fetal development, or the expression in adults may be masked by dense SP innervation.

These data show that substance P is expressed in the target nuclei of the NS at about the same time that NS axons probably reach these targets. The early expression and development of this peptide suggests that substance P may serve as a trophic factor during the formation of the BG. Synaptogenesis may permit its subsequent function as a neurotransmitter/neuromodulator in adults. Supported by USPHS RR05756, HD05958 and AG01558.

435.6 FINE STRUCTURE OF THE OPOSSUM SUBSTANTIA NIGRA PARS LATERALIS. T.P. Ma and J.C. Hazlett. Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI. 48201.

The substantia nigra pars lateralis (SNl) of adult opossums (Didelphis virginiana) was examined with the electron microscope. Small cells (≤ 18 um long axis) had round nuclei with clumps of heterochromatin and prominent nucleoli. A thin rim of cytoplasm contained all commonly observed neuronal organelles except Nissl bodies. Medium to large cells (> 18 um long axis) possessed round to oval nuclei with prominent nucleoli. A few of these nuclei exhibited indentations. All common neuronal organelles were observed, including prominent Nissl bodies. These were particularly prominent at juxtanuclear positions opposite the emergence of dendrites. Few synaptic profiles were observed to contact the somata, however, dendrites were usually ensheathed by endings. Five types of presynaptic profiles were observed. Types A and B were asymmetric while Types C, D, and E were symmetrical. Type A terminals contained oval to round clear vesicles (40-60 nm), a few large dense core vesicles, and mitochondria. Type B endings possessed oval to round clear vesicles (20-40 nm) but lacked dense core structures. Both Type A and B terminals contacted dendrites of all sizes which always exhibited prominent postsynaptic thickenings with subjunctional bodies. Type C endings had equal pre- and postsynaptic thickenings. The presynpatic profile contained round to oval clear vesicles (40-60 nm), dense core vesicles, and mitochondria and contacted dendrites and spines. Type D endings were large, contain many mitochondria and only contacted proximal dendrites and cell bodies. The vesicles were small and pleiomorphic and accumulated adjacent to numerous short active zones. The type E synapses possessed a "core" region which had a crystalline-like array of vesicles (35 nm) and a "peripheral" region which contained round to oval vesicles (40-60 nm), large dense core vesicles and mitochondria. These endings which contacted dendrites and somata exhibited multiple presynaptic thickenings. Injections of HRP into the superior colliculus labeled medium to large sized neurons, and large and intermediate sized dendrites. All five types of presynaptic profiles contacted the labeled neuronal elements. These results are similar to those described for the substantia nigra pars reticulata (SNr) in other species at the EM level. The principle differences are (1) the morphology of the nuclear envelope and (2) the presence of a "core" region in type E profiles. This lends support to the hypothesis that the SNl and SNr may subserve similar functions in the modulation of basal ganglia output.

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435.7 LARGE PHA-L LABELED PALLIDAL TERMINALS FORM SYMMETRICAL SYNAPSES ON THE SOMATA AND DENDRITES OF SUBTHALAMIC AND SUBSTANTIA NIGRAL NEURONS. H. Kita and S.T. Kitai, Department of Anatomy and Neurobiology, The University of Tennessee, Memphis, Collage of Medicine, Memphis, TN 38163.

Projections from the globus pallidus (GP) to the subthalamic nucleus (STH) and the substantia nigra (SN) in rats were studied using Phaseolus vulgaris Leucoagglutinin (PHA-L) anterograde tracing method. PHA-L was iontophoretically injected into the GP of anesthetized rats. After two weeks of survival, rats were perfused transcardially with acetate-borate buffered solutions containing 4% paraformaldehyde and 0.15% glutaraldehyde. Parasagittal or horizontal sections (40 μm) were cut using a Vibratome. The sections were processed for immunohistochemical visualization for PHA-L using avidin-biotin-peroxidase method with diaminobenzidine (DAB). Some sections were processed for dual labeling of PHA-L and tyrosine hydroxylase (TH). In this case, PHA-L was first visualized by peroxidase-anti-peroxidase method with DAB (brown reaction) and then TH was localized by avidin-biotin- peroxidase method with cobalt-DAB (blue reaction).

Light microscopic observation revealed that the PHA-L labeled GP afferent plexus in the STH and SN consisted of thin fibers with boutons en passant and terminaux. Most terminals were large (over 1μm). In SN, PHA-L labeled terminals surrounded not only TH immunoreactive neurons but also non-reactive neurons.

Electron microscopic analysis revealed that the morphological features of the labeled processes were very similar in the STH and SN. Labeled fibers were either myelinated or unmyelinated. Labeled terminals were large and contained many small spherical or ellipsoidal vesicles and a number of mitochondria. They formed symmetrical synapses mainly with somata and large diameter (more than 1μm) dendritic shafts.

The present findings demonstrate the anatomical basis for the known powerful inhibitory pallidal projection to the STH. They also show that the pallidal projection to SN is not limited to dopaminergic neurons, and suggested that the function of this pathway may be inhibitory. If so, the density of this projection, and its terminal distribution on the proximal regions of the cells indicate that it may be a major influence on the firing of SN neurons.

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435.8 SUBSTANTIA INNOMINATA AND PEDUNCULOPONTINE TEGMENTAL NUCLEUS IN THE RAT: EVIDENCE FOR PREDOMINANTLY NONCHOLINERGIC RECIPROCAL INNERVATION. L.J. Martin*†, V.E. Koliatsos†, H.J.W. Nauta§, M.R. DeLong§§ and D.L. Price† (SPON: M.R. DeLong). †Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; §Dept. of Neurosurgery, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; §§Neurophysiology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Direct projections from the substantia innominata (SI) to dorsal mesencephalic tegmentum, specifically the pedunculopontine tegmental nucleus (PPN), have been described recently, but the detailed organization and transmitter specificity of this pathway have not been fully documented. Moreover, the relationship of tegmentopetal SI neurons with cholinergic SI neurons that invade this forebrain area is not understood. These experiments were designed to investigate patterns of connectivity between neurons in the sublenticular substantia innominata (SLSI) and ventral pallidum (VP) and neurons in the PPN. First, the anterogradely transported lectin Phaseolus vulgaris leukoagglutinin (PHA-L) was iontophoresed into VP. Descending fibers followed either a ventral course through the caudal ventrotegmental area and retrorubal fields or a dorsal course through ventromedial central gray; these fiber pathways united at the level of the decussation of the superior cerebellar peduncle (SCP) and terminated massively in the PPN, particularly in the paratrochlear region. Second, implants of fluorescent tracer Fluoro Gold (FG) were placed in the PPN at the level of the decussation of SCP. Retrogradely labeled neurons were consistently observed in the ventral and dorsomedial pallidum, SLSI, and nucleus interstitialis of the ansa lenticularis. Combined retrograde axonal tracing and Texas Red-choline acetyltransferase (ChAT) immunofluorescence revealed that these tegmentopetal neurons are noncholinergic. Third, recognizing the reciprocity of PPN connections with compartments of the basal ganglia, we tested the hypothesis that PPN and SI may have reciprocal connections. FG implants were made in SLSI and VP; retrogradely labeled neurons were present in the PPN (pars dissipatus and pars compactus). Texas Red-ChAT immunofluorescence showed that ca. 20% of the total number of labeled neurons were cholinergic and were located in pars compactus, closely associated with the ventrolateral border of the decussation of the SCP and ascending limb. Retrogradely labeled neurons were also present in the laterodorsal tegmental nucleus (LDTg), but <10% of these neurons were ChAT positive. These findings indicate that the PPN has a reciprocal connection with the SI and VP, parts of the basal forebrain related to basal ganglia limbic circuitries. Moreover, cholinergic neurons of the SI and PPN do not appear to make substantial contributions to these circuits.

435.9 ULTRASTRUCTURE OF THE NUCLEUS TEGMENTI PEDUNCULOPONTINUS IN THE RAT. B.M. Spann and I. Grofova. Dept. of Anatomy, Michigan State University, E. Lansing, MI 48824.

Numerous light microscopic studies have focused on the interrelationships between the nucleus tegmenti pedunculopontinus (PPN) and the basal ganglia (BG), and several hypotheses on the functional significance of these connections have been postulated. To date, there exist neither electron microscopic confirmation of the termination of the BG efferents within the PPN nor normal baseline data on the ultrastructural organization of this nucleus. The present study describes normal ultrastructural features of the rodent PPN and presents experimental data on the morphology and termination of the PPN afferents from the substantia nigra.

The ultrastructural analysis was performed in well-defined samples selected from sagittal sections through the lateral, intermediate and medial portions of the PPN. The observations suggest that the PPN afferents terminate almost exclusively on the dendrites since the synaptic terminals on the cell bodies are extremely rare. The neuropil is characterized by an abundance of myelinated fibers of varying diameters, small bundles of unmyelinated fibers and a lattice of dendrites which are rather sparsely covered by nerve terminals. Three morphologically distinct types of terminal boutons have been observed so far. The most common are small (<1 µm) Type I terminals which contain densely packed round synaptic vesicles and establish asymmetrical synapses with both thick and thin dendrites. The Type II boutons are larger and less frequent than Type I. They contain round synaptic vesicles which tend to be arranged in clusters and are involved in asymmetrical synapses which are characterized by the presence of a regular array of presynaptic densities and postjunctional dense bodies beneath the post-synaptic density. In addition to the synaptic junction, they often exhibit one or more puncta adherens. Type III terminals contain clusters of small pleomorphic vesicles and several mitochondria in a clear matrix. They form symmetrical synaptic junctions predominantly with medium-size dendrites. In one instance, the Type III terminal was seen synapsing on the cell body in the medial region of the PPN. While the three bouton types are present throughout the nucleus, the Type III terminals are more frequently encountered in the samples from the intermediate and medial portions of the nucleus.

The origin of the Type I and Type II terminals is at present unknown. Concerning the Type III terminals, our experimental evidence indicate that they are at least partially of nigral origin since boutons of similar morphology were labeled following iontophoretic injections of Phaseolus vulgaris-leucoagglutinin(PHA-L) into the substantia nigra pars reticulata.

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435.10 DISTRIBUTION OF EFFERENT CONNECTIONS OF THE SUBSTANTIA NIGRA PARS RETICULATA IN THE RAT: ANTEROGRADE ANALYSIS UTILIZING PHA-L. I. Grofova and B.M. Spann, Dept. of Anatomy, Michigan State University, E. Lansing, MI 48824.

Several lines of evidence have indicated that the pars reticulata of the substantia nigra (SNR), which represents one of the output nuclei of the basal ganglia, is involved in the control of various aspects of motor behavior. These effects are presumably mediated through the nigro-thalamocortical, nigrotectal and nigroreticular pathways. Major goal of the present study was to accurately define the targets of nigral efferents using the anterograde tracer Phaseolus vulgaris-leucoaggulutinin (PHA-L) which provides labeling of single fibers, terminal plexuses and varicosities.

Single or multiple iontophoretic injections of PHA-L were made in the SNR through micropipettes using stereotaxic coordinates of Paxinos and Watson (1986). Following survival periods of 10-14 days, the animals were perfused intracardially by a fixative consisting of 4% paraformaldehyde and 0.2% glutaraldehyde in 0.15M phosphate buffer. The brains were cut in 30 µm thick sagittal sections on a vibratome and reacted immunohistochemically using a modified protocol by Gerfen and Sawchenko (Brain Res. 290:219-238, 1984).

Examination of the light microscopic material confirmed previous reports of SNR projections to the ventromedial and intralaminar thalamic nuclei, superior colliculus, central gray, nucleus tegmenti pedunculopontinus (PPN) and midbrain reticular formation, and in addition, revealed several hitherto unknown targets of nigral efferents. In the thalamus, a particularly dense terminal plexus was seen in a crescent-shaped area in the rostral portion of the nucleus ventralis lateralis. Furthermore, dense patches of labeled terminal fibers were consistently present in the dorsal part of zona incerta including the pars magnocellularis which is known to project to the spinal cord. In the midbrain, fine labeled fibers exhibiting terminal varicosities were observed around large and smaller neurons in the red nucleus. Terminal plexus was also noticed in the nuclei related to the medial longitudinal fasciculus, particularly in the rostral interstitial nucleus. In the pons, most of the nigral fibers terminated in the PPN and only a modest number extended to the ventral parabrachial nucleus, subcoeruleus and nucleus pontis oralis.

This study shows that the distribution of SNR efferents is more widespread than generally thought. Nigral projection to the red nucleus is of particular interest since it may provide a direct route for the basal ganglia control of the bulbar and spinal motor systems.

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435.11 CHOLECYSTOKININ-IMMUNOREACTIVE NEURONS IN THE NIGROSTRIATAL SYSTEM OF RATS. C.E. Adams, Dept. Cellular and Structural Biology, Univ. Colo. Hlth. Sci. Ctr., Denver, CO. 80262.

The rat neostriatum (NS) contains high levels of the neuropeptide cholecystokinin (CCK). Some striatal CCK is produced by a small population of intrinsic aspiny neurons. The majority of the CCK, however, originates from hitherto unidentified regions that project into the NS. I have determined, utilizing a combined retrograde transport/immunocytochemical technique, that the substantia nigra (SN) is one source of CCK for the rat NS. Three rats received intrastiatal injections of WGA/HRP (2%, 0.01-0.05ul). After 48 hours, the rats were given intracisternal injections of colchicine (8-14ul, 9ug/ul). Thirty-six hours later, the rats were perfused transcardially with 0.9% saline followed by 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4), the brains removed and stored overnight in buffered 20% sucrose (pH 7.4). Frozen sections (30-50um) through the SN and NS were incubated in tetramethylbenzidine (TMB) with nitroferricyanide. After a brief saline wash, the sections were incubated in diaminobenzidine (DAB) with nickelous ammonium sulfate and cobalt chloride. This double incubation produces a punctate blue/black reaction product in the neurons containing HRP. The SN sections were subsequently stained for CCK by the indirect peroxidase anti-peroxidase method (CCK antiserum at 1:3000; CRB Inc.; 48 hrs, 4° C). CCK-immunoreactive (CCK-IR) neurons, as identified by a diffuse red-brown color, were found at all levels of the SN, primarily within the pars compacta (SNc), but also occasionally within the pars reticulata (SNr). The distribution of the CCK-IR cells was highest within the medial and lateral thirds of the SNc and lowest within the middle third. Neurons retrogradely labeled with WGA/HRP were seen within the ventral tegmental area and medial third of the SNc following two injections into the medial striatum/lateral accumbens. An injection placed more laterally in the striatum retrogradely labeled cells within both the medial and middle thirds of the SNc as well as within the middle third of the SNr. Between 25 and 75% of the retrogradely labeled SNc neurons were immunoreactive for CCK. These data indicate the SNc is a source for striatal CCK as well as dopamine (DA). The potential colocalization of DA and CCK within a subpopulation of rat nigrostriatal neurons is currently being investigated. Since the level of CCK within the SN of Parkinsonian patients has been reported to be below that of normal controls, alterations in the regulation of CCK within the SNc may contribute to the deficits of Parkinson's disease.

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435.12 AFFERENT CONNECTIONS OF THE SUBTHALAMIC NUCLEUS OF LUYS, STUDIED IN THE RAT BY ANTEROGRADE AND RETROGRADE TRANSPORT OF HRP. N.S. Canteras*, S.J. Shammah-Lagnado*, B.A. Silva* and J.A. Ricardo. Dept. of Physiology and Biophysics, Institute of Biomedical Sciences of the University of São Paulo, 01000 São Paulo, SP, Brazil.

Previous anatomical data indicate that the subthalamic nucleus of Luys (STN) projects massively to the external and internal segments of the globus pallidus and to the pars reticulata of the substantia nigra (for the rat, see Ricardo, J.A., Brain Res., 202: 257, 1980). This fact suggests that the STN is in a strategic position to modulate important extrapyramidal neural mechanisms, and points to the interest of a thorough knowledge of the STN afferent connections. In the present study, a systematic examination of the STN afferents was undertaken in the rat by the aid of retrograde and anterograde tracer techniques. The markers, either free horseradish peroxidase (HRP) or HRP conjugated with wheat germ agglutinin (WGA-HRP), were microelectrophoretically ejected and the tissue sections processed according to the tetramethylbenzidine method.

Our results, besides confirming the existence of previously reported pathways, point to several cortical and subcortical territories as the sources of hitherto undescribed projections to the STN. Thus, whereas the external segment of the globus pallidus seems to represent the main origin of the afferent connections of the STN, a substantial input to the latter nucleus appears to come also from the motor, somatosensory and medial prefrontal districts of the cerebral cortex, as well as from the thalamic parafascicular nucleus and the nucleus tegmenti pedunculopontinus, pars compacta. Several other structures, among which the granular insular, visual and retrosplenial cortices, the ventral pallidum, the central amygdaloid and anterior pretectal nuclei, the deep layers of the superior colliculus, the lateral nucleus of the cerebellum, the nuclei raphe dorsalis and magnus, the spinal trigeminal nucleus, pars oralis, and the dorsal column nuclei, seem to represent modest but consistent additional sources of projections to the STN.

The new findings of the present study suggest, among other implications, that the STN would be the recipient of direct projections from the cerebellum and widespread cortical territories and of a varied and potentially significant sensory input.

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435.13 AFFERENT CONNECTIONS OF THE CAUDATE NUCLEUS IN THE CAT. Z.H. Zhang* and A.C. Rosenquist. Department of Anatomy, Univ. of Pennsylvania, Philadelphia, PA 19104-6058.

Cortical and subcortical afferents to the caudate nucleus of 6 cats were studied by charting the locations of retrogradely labeled cells after injection of a tiny pellet of WGA-HRP embedded in polyacrylamide gel into different regions of this nucleus. The use of the solid gel prevented all leakage of tracer into the needle track. The HRP was visualized using the standard TMB protocol after a 48 h survival time.

Injection sites were localized in either the dorsolateral (DL) or ventromedial (VM) head of the caudate from A17 to A15 (N=5) or the dorsal posterior (DP) portion of the caudate at A13.5 (N=1). The following ipsilateral cortical areas contained retrogradely labeled cells after all 6 injections and will be referred to as "common" areas: frontal eye fields (FEF), cingulate gyrus, area 7, the splenial visual area, and visual areas 20a and 20b. In most, but not all cases these common areas were labeled on the contralateral side as well; and always to a lesser degree than ipsilaterally. Injections restricted to the DL head of the caudate resulted in the following additional label: areas 5 and 19 ipsilaterally; and PMLS and AMLS bilaterally. By contrast, injections restricted to the VM head of the caudate resulted in different additional label in the ectosylvian visual area, area 4 and AI and AII ipsilaterally; and in the insular cortex and PLLS and ALLS bilaterally. The most caudal injection into the DP caudate resulted in label in the common areas plus area 19 ipsilaterally. In all cases the cells of origin of the corticostriatal projection arose from both the supra and infragranular cortical layers. Approximate percentages of the corticostriatal cells in the FEF were: layer II 6%, layer III 58%, layer V 29%, layer VI 7%.

In all 6 cases subcortical label was present ipsilaterally in the intralaminar nuclei of the thalamus, the subthalamic nucleus, the retrorubral fields and bilaterally in the medial 1/2 of the substantia nigra (pars compacta and reticulata) and the dorsal raphe nucleus. Labeled cells in the amygdala were seen ipsilaterally only after injection into the VM head of the caudate.

Our results support the following conclusions. First, some cortical areas project to the entire head of the caudate (common areas), whereas some project only to the DL or VM portions. Second, corticostriatal cells are located in the supra- as well as infragranular layers (Royce,G.J.,J.comp.Neurol.,205:8-29, 1982).

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435.14 POTENTIAL EVIDENCE FOR A NEW SUBDIVISION IN THE NEOSTRIATUM OF THE RAT. S.Y. Shu* and G.R. Penny. Department of Anatomy and Cell Biology, School of Medicine, East Carolina University, Greenville, NC 27858

In horizontal or parasagittal Nissl sections through the rat neostriatum a caudal band of densely packed medium-sized cells can be seen lying at the border separating the neostriatum from the globus pallidus. This cell group, referred to here as the strio-pallidal border zone, is shaped like a flattened plate, occupying almost the entire dorsoventral and mediolateral extent of the striatal border with the external medullary lamina. Often it is broken into islands of cells by the perforating internal capsule fascicles.

A projection from the striatum to the border zone was previously described by Wilson and Phelan (1982); in the present study, wheat germ agglutinin conjugated to HRP (WGA-HRP) was used combined with histochemical staining for acetylcholinesterase (AChE) to further examine the connections, histochemical characteristics and internal organization of the border zone. Iontophoretic injection of WGA-HRP within the neostriatum rostral to the border zone reveals a narrow band of anterogradely labeled terminals within the border zone in addition to projections to the more caudal globus pallidus and substantia nigra. Double labeling of some sections for AChE suggests that the band of labeled terminals is within the AChE-rich striatum. Scattered retrogradely labeled cells are also seen within the field of labeled terminals, indicating that the few neurons in the border zone give rise to reciprocal projections to the main body of the striatum.

Small injections of tracer within the border zone reveals (1) retrogradely labeled cells elsewhere in the striatum, (2) widespread anterogradely labeled terminals extending millimeters from the injection site but confined within the border zone, and (3) projections to the globus pallidus and substantia nigra.

These results indicate that the border zone on grounds of position, cytoarchitecture and histochemistry might be a part of the neostriatum, but that nevertheless it has a markedly different connectional organization from the rest of that structure. Unlike the main body of the striatum, the border zone receives afferents from the rest of striatum, contains cells that give rise to an extremely widespread system of local collaterals, and contains cells that project back to the main body of the striatum; like the rest of the striatum the border zone projects to the globus pallidus and substantia nigra.

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435.15 PROJECTIONS OF VISUAL CORTICAL AREAS VIA THE NEOSTRIATUM TO THE GLOBUS PALLIDUS AND SUBSTANTIA NIGRA IN THE RHESUS MONKEY. J.A. Saint-Cyr, L.G. Ungerleider and R. Desimone. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

Injections of multiple anterograde and retrograde tracers were made into the tail and genu of the caudate nucleus or adjacent ventral putamen under physiological control in five rhesus monkeys.

After caudate injections, labeled cells were found both in a large continuous region of cortex topographically related to the site of injection, and in several non-contiguous cortical regions. After injections in the rostral tail, the continuously labeled region included rostral area TE and adjacent portions of TF, TH, TG, and, occasionally, area 35. After injections into the posterior tail and ventral genu, the labeled region shifted posteriorly to posterior TE, TF, and TEO and then into prestriate areas V4, MT, PO, and (sparsely) V3 and V2. As the injection site advanced into the dorsal genu and then to the caudal body, the labeled region shifted toward the parietal lobe, to area 7, to areas VIP and LIP in the intraparietal sulcus, and into area 5 and adjacent area 23.

The non-contiguous areas labeled by nearly all injections included the principal sulcus/frontal eye field region, area 24, the superior temporal polysensory area, and, more rarely, area 25. Thus, whereas certain temporal, occipital, and parietal cortical areas project into the neostriatum largely according to proximity, as originally shown by Kemp and Powell, prefrontal, anterior cingulate, and superior temporal sulcal areas have a wider distribution, consistent with more recent studies.

Labeled cortical cells were mostly in layer 5 but also in layer 3. Labeled subcortical cells were found in the amygdala, thalamic nuclei centrum medianum and parafascicularis, globus pallidus (GP), substantia nigra pars compacta, and the ventral tegmental area.

Efferent projections were confined to GP and substantia nigra pars reticulata (SNr). Whereas the ventral genu and entire tail of caudate and adjacent putamen projected to ventral GP at the border between its internal and external subdivisions, the dorsal genu and adjacent body of caudate projected to GP at its dorsal border. Both ventral and dorsal portions of the neostriatum projected to the rostrolateral SNr in a partially overlapping fashion.

Because of the known projections from both GP and SNr to the frontal lobe via the thalamus and from SNr to the superior colliculus, the neostriatum could provide alternate routes by which visual cortical areas could influence dorsolateral prefrontal and collicular regions to which they project directly. The latter regions are thought to have spatial and/or visuomotor functions, and, in this regard, it is interesting that the portions of the caudate receiving visual inputs also receive inputs from polysensory areas implicated in these functions.

435.16 RECIPROCAL CONNECTIONS BETWEEN THE NUCLEUS ACCUMBENS AND THE VENTRAL PALLIDUM IN THE RHESUS MONKEY. C. Weiss and S.N. Haber. Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642

The ventral pallidum of the rat receives its input from the nucleus accumbens while the dorsal pallidum receives projections from the caudatoputamen (Heimer et al, Trends Neurosci. 5:83). Enkephalin-positive woolly fibers are found throughout the globus pallidus and the ventral pallidum; substance P-positive woolly fibers are concentrated in the ventral pallidum and has been suggested as a marker for the ventral pallidum (Haber and Nauta, Neurosci. 9:245). The distribution of enkephalin, and substance P-positive woolly fibers were recently described in the human globus pallidus (Haber and Watson, Neurosci. 14:1011). Dense enkephalin-like immunoreactivity is observed throughout the external segment and in the ventral pallidum. Substance P-like immunoreactivity is more limited in the pallidal region and is found in a thin semi-circular band ventral to the anterior commissure but not directly beneath it and extending dorsomedially into the rostral pole of the external segment. This pattern is similar to monkeys but unlike the ventral pallidum of the rat where substance P-positive fibers show a more extensive ventral subcommissural distribution but does not extend into the dorsal pallidum. It raises the question of what area in the primate forebrain is homologous with the ventral pallidum of the rat. From the staining patterns it might be inferred that the area defined as the dorsal pallidum in the rat and receiving input from the caudate nucleus rather than from the nucleus accumbens is, in primates larger, and extends beneath the anterior commissure, thereby pushing the ventral pallidum, or that region which receives input from the nucleus accumbens, ventralward as well as dorsomedially into the rostral pole of the external segment.

We report here an autoradiographic and WGA-HRP study of the projections from the nucleus accumbens in the rhesus monkey with comments on the pallidal and nigral inputs. Control experiments involve the dorsal striatum. Preliminary findings indicate that the nucleus accumbens, in primates, projects to a region ventral to the anterior commissure but not directly beneath it. Woolly fibers formed by these terminal fields are also observed in the rostral pole of the external segment of the globus pallidus but are not found in more caudal regions of that segment. This projection appears to be reciprocal as labeled WGA-HRP cells are located among the terminal fields in these pallidal regions. This projection from the nucleus accumbens appears to coincide well with the distribution of substance P-positive woolly fibers.

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435.17 CELLS IN THE LATEROPOSTERIOR THALAMIC NUCLEUS AND SUBSTANTIA NIGRA PARS LATERALIS MAY INTEGRATE THE VISUAL SYSTEM WITH THE BASAL GANGLIA.

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The lateroposterior thalamic nucleus (LP) is the major source of visually related thalamostriatal projections. The LP also sends fibers to the secondary visual cortex and in turn receives inputs from the secondary visual cortex and superior colliculus (SC) and further from the substantia nigra pars lateralis (SNl). In addition to its LP efferents, the SNl sends axons to the SC and amygdala and further to the striatum. These facts prompted morphological approaches to integrative mechanisms between the visual system and basal ganglia. The first goal of this study was to examine whether or not single LP neurons give rise to divergent collateral projections to the visual cortex and striatum, and whether or not single SNl neurons give off axonal branches to the LP/SC and striatum. The data from our retrograde fluorescent double labeling experiments (with true blue and diamidino yellow) in the rat clearly show that a small population of LP cells projecting to the striatum have axon collaterals to the visual cortex, whreas a large population of single SNl cells project to both the LP/SC and striatum,

Furthermore, considerable histochemical studies have previously suggested that the SNl cells contain dopamine as a possible neurotransmitter. The second goal of our experiments was to identify if the SNl cells projecting to the LP, SC, striatum and/or amygdala contain dopamine. Employing a combined retrograde fluorescent tracing (with fluoro-gold) and immunofluorescent histochemistry for tyrosine hydroxylase, the synthesizing enzyme for dopamine, we show that subpopulations of SNl cells sending fibers to each target are presumably dopaminergic, although the proportion of dopaminergic cells in each group can vary from more than 80% (to the amygdala) to less than 10% (to the LP and SC).

In conclusion, cells in the LP and SNl might provide an important role in integrating visual and motor systems.

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435.18 THE SUBPARAFASCICULAR NUCLEUS: AN ORIGIN OF THALAMIC COLLATERAL DOPAMINE PROJECTIONS TO THE NEOCORTEX AND SPINAL CORD.

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Very little has so far been known with certainty about the neuronal characteristics of the subparafascicular nucleus (Spf) of the thalamus, except that the nucleus, which may probably be posited in the A11 catecholaminergic cell group, projects to the amygdala, inferior olive and spinal cord. In the present study, the organization of projection neurons in the Spf was examined in the rat with a combined retrograde labeling (with fluoro-gold)- immunohistochemical method for the demonstration of dopaminergic neurons, and with retrograde fluorescent double labeling method. Seven brain areas were tested here as output targets of the Spf: the neocortex (frontal/prefrontal cortices), striatum, nucleus accumbens, amygdala, subthalamic nucleus, inferior olive and spinal cord (upper cervical segments). First, the distribution of dopaminergic and non-dopaminergic Spf cells that project to each terminal field was determined. Second, various combinations of injections of two tracers (fluoro-gold and propidium iodide or true blue and diamidino yellow) into different terminal fields were made to determine whether single cells in the Spf send divergent axon collaterals to more than one target.

The results indicate that essentially separate groups of cells in the Spf project to each terminal field, although subpopulations of Spf cells give off axonal branches to the spinal cord and one of telencephalic structures including the neocortex, striatum, nucleus accumbens and amygdala. Furthermore, each group can be divided into dopaminergic and non-dopaminergic components, although the proportion of dopaminergic cells in each group can vary from more than 80% (to the spinal cord) to less than 10% (to the subthalamic nucleus and inferior olive). Of particular interest is that a distinct population of dopamine-containing Spf cells project simultaneously to both the neocortex and spinal cord. These bifurcating dopaminergic projection neurons in the Spf might play a unique role in somatic motor coordinations. Thus, the Spf with widespread dopamine projections may be in a strategic position to exert a prominent control over somatic motor- and limbic-related functions.

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435.19 PALLIDAL PROJECTIONS TO SUBTHALAMUS, SUBSTANTIA NIGRA AND PEDUNCULO-PONTINE NUCLEUS: EVIDENCE FOR COLLATERALIZATION OF PALLIDAL PROJECTIONS REVEALED BY WHEAT GERM-HRP HISTOCHEMISTRY COMBINED WITH CHOLERA TOXIN B IMMUNOCYTOCHEMISTRY.

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Projections of the globus pallidus (GP) to the subthalamic nucleus (STH), the substantia nigra (SN) and the pedunculopontine nucleus (PPN) were studied using retrograde axonal transport of wheat germ-HRP (WGA-HRP) and cholera toxin B (CTB).

Rats were anesthetized with a ketamine-xylazine mixture (i.p.,100 mg/Kg). WGA-HRP (0.1-0.15 µl 1% in saline) and CTB (0.15 µl 1% in distilled water) were injected using pressure technique. Two sites were always selected for injection per animal. In STH-SN or STH-PPN combinations, WGA-HRP was injected in STH and CTB in SN or PPN. In SN-PPN combination, WGA-HRP was injected in SN and CTB in PPN. After two days of survival time, the rats were perfused with a mixture of 3% paraformaldehyde and 0.2% glutaraldehyde in 0.15 M phosphate buffer pH 7.35. For the histological processing, the brains were parasagittally sectioned on a Vibratome. The sections were first processed for WGA-HRP using DAB-nickle method which resulted in a blue-black punctate reaction product in GP projection neurons. Subsequently, the sections were processed for CTB with o-dianisidine, which resulted in a green reaction product or DAB using a glucose oxidase-coupled method for a brown reaction product. Sample counts showed that 79-82% of GP neurons projected to STH; 46-60% to SN and 43-52% to PPN. Twenty-five to 46% of GP neurons were double labeled after STH and SN injections, 4-14% double labeled after STH and PPN injections and 12-22% double labeled after SN and PPN injections. Even though pallido-STH and pallido-SN projection neurons were intermingled throughout GP, pallido-SN projection neurons tended to be located in the ventral part of GP. The pallido-PPN neurons were found in the lateral and ventral portion of GP. The morphology of the pallidal projection neurons, regardless of their projection sites, was similar. CTB appeared to be more sensitive than WGA-HRP in terms of uptake, transportation,and visualization.

The present results indicate that 1) allidal projections to STH, SN and PPN are achieved by the axon collaterals of pallidal neurons, 2) there is some topographical organization of pallidal projection neurons, and 3) CTB is a sensitive axonal tracer that can be used alone or combined with other tracers.

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435.20 IDENTIFIED CORTICOSTRIATAL SYNAPSES INVOLVING NADPH-DIAPHORASE POSITIVE AND NEUROPEPTIDE Y IMMUNOREACTIVE NEURONS IN THE RHESUS CAUDATE NUCLEUS.

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Excitotoxin models suggest that striatal degeneration in Huntington's disease (HD) may be related to glutamatergic corticostriatal input. Recent studies have shown that in HD aspiny neurons containing NADPH-diaphorase (NADPH-d), somatostatin, and neuropeptide Y (NPY) are selectively spared while spiny neurons are severely involved. Since dendritic spines are the major site of termination of corticostriatal efferents, the aspiny neurons may be spared because of a paucity of corticostriatal synapses. This is an electron microscopic study of NPY and NADPH-d containing neurons in the rhesus monkey and of their synapses with degenerating corticostriatal axon terminals.

Areas of frontal cortex were aspirated in several adult rhesus monkeys. After 5 days they were perfused and the ipsilateral basal ganglia were removed and vibratome-sectioned at 100 µm. Adjacent sections were stained with the Fink-Heimer method and either by NADPH-d histochemistry of NPY immunocytochemistry. Labeled neurons located in zones of terminal degeneration as indicated by adjacent Fink-Heimer sections were serially thin-sectioned for electron microscopy. Extensive portions of these neurons were reconstructed to demonstrate synapses with degenerating corticostriatal axon terminals. Somata receive several types of axon terminals making symmetrical or asymmetrical synapses. Dendrites also form both types of synapses. The asymmetrical type is far more frequent beyond the most proximal dendritic segments. Most of the identified neurons were located within neuropil containing degenerating corticostriatal axon terminals which formed asymmetrical synapses with spines, dendritic shafts and somata. Only occasionally did these synapses involve the dendrites or somata of the identified neurons.

The survival of aspiny neurons in HD may, in part, be due to receiving few corticostriatal synapses. Quantification of the corticostriatal synapses of aspiny and spiny caudate neurons will be required to better assess this possibility.

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436.1 ORGANIZATION OF STRIATAL AND SUBTHALAMIC NUCLEUS AFFERENTS TO PALLIDUM IN PRIMATE AS REVEALED BY PHA-L ANTEROGRADE TRACING METHOD. L. Hazrati*, A. Parent and Y. Smith (SPON: R. Boucher). Lab. of Neurobiology, Fac. of Med., Laval Univ., Québec, Canada.

The lectin Phaseolus vulgaris-leucoagglutinin (PHA-L) was used as an anterograde tracer to study the patterns of termination of striatopallidal and subthalamopallidal fibers in the squirrel monkey (Saimiri sciureus). Small iontophoretic injections of PHA-L in the subthalamic nucleus (ST) led to profuse fiber labeling in the ipsilateral globus pallidus (GP). The coarse and smooth labeled fibers that arose from the injection site traversed the internal capsule via the subthalamic fasciculus, invaded the caudomedial pole of the internal pallidal segment (GPi) and extended rostrally throughout GP. In the caudal third of GP, most labeled fibers occurred in the inner part of GPi where they formed a rich plexus. The outer part of GPi contained a moderate number of labeled fibers while only a few fibers were scattered in the external pallidal segment (GPe). In the middle third of GP, labeled fibers abounded along the inner and outer surfaces of the internal medullary lamina where they displayed a typical band-like pattern. In addition, a small to moderate number of fibers arborizing in all directions were present in the core of GPi and GPe. In the rostral third of GP, fibers abounded in GPe where they formed two distinct bands; one in the central core, the other along the external medullary lamina. These two bands were interconnected by numerous fibers oriented mediolaterally. On the other hand, small PHA-L injections in the striatum led to intense labeling of typical medium-sized spiny neurons close to the injection site as well as numerous, very thin and unvaricosed fibers projecting outside the striatum. Following injection in the caudate nucleus, labeled fibers traversed the internal capsule and coursed along the dorsal third of GPe and GPi where they formed several small plexuses that were not topographically related to medullary laminae. PHA-L injection in the putamen resulted in a massive anterograde labeling of fibers in ventral two-thirds of GP. Rostrally, labeled fibers formed several interrelated plexuses in GPe, whereas in the middle third of GP, most fibers were regrouped into two distinct bands lying along inner and outer surfaces of the internal medullary lamina. At caudalmost levels of GP, fibers were mostly confined to the medial pole of GPi where they arborized profusely in all directions. The fibers within typical bands observed at various levels of GP after striatal or ST injections were characteristically thin and markedly varicosed. Our results reveal that both striatal and subthalamic nucleus afferents to the pallidum in primates display strikingly complex band-like patterns that appear to be in register with one another.

436.2 DOPAMINERGIC INNERVATION OF PALLIDUM AND SUBTHALAMIC NUCLEUS IN PRIMATE. Y. Smith and A. Parent. Lab. of Neurobiology, Fac. of Med., Laval Univ., Québec, Canada.

An antiserum raised against tyrosine hydroxylase (TH) was used to study the dopaminergic innervation of the basal ganglia in the squirrel monkey (Saimiri sciureus). Complete series of transverse and horizontal sections of midbrain and basal forebrain were incubated with this TH antiserum, whereas some alternate sections were also incubated with a dopamine- ß -hydroxylase (DBH) antiserum. The dopaminergic nature of neuronal elements described in the present study was ensured by the fact that they all stained positively for TH but not for DBH. Axons of midbrain TH-immunoreactive cell bodies accumulated mediodorsally to the substantia nigra pars compacta where they formed a massive bundle ascending through the field H of Forel. The dopaminergic innervation of the globus pallidus (GP) derived mostly from two fascicles that detached themselves from this main bundle and reached their target by coursing along the two major output pathways of GP: the lenticular fasciculus caudally and the ansa lenticularis rostrally. Some TH fibers coursing along the lenticular fasciculus arborized within the dorsomedial third of the subthalamic nucleus, while most traversed the internal capsule and invaded GP from its dorsal surface. More rostrally, a larger contingent of TH fibers swept laterally along the ansa lenticularis to invade GP from its ventral surface. These fibers ascended within medullary laminae and arborized profusely within both internal (GPi) and external (GPe) pallidal segments. In the caudal third of GP, numerous thick and smooth TH fibers occurred in the dorsal portion of each segment where they arborized in all directions. In the middle third of GP, TH fibers were significantly more numerous in GPi than in GPe. In GPi, they formed numerous small plexuses distributed over the entire dorsoventral extent of the segment, whereas GPe displayed only few short fibers that were uniformly scattered throughout its dorsal portion. In the rostral third of GP, TH fibers remained more abundant in GPi than in GPe, but the rostral pole of GPe contained a significant number of fibers forming small compact fascicles oriented dorsolaterally toward the putamen. Other TH fibers were seen to leave the main bundle at various levels of the lateral hypothalamus to ascend along the lateral border of the reticular thalamic nucleus or to continue rostrally toward the olfactory tubercle and the head of the caudate nucleus. Our results reveal that the dopaminergic innervation of the subthalamic nucleus in primates is relatively weak and confined to its dorsomedial third. In contrast, the pallidum receives a massive dopaminergic afferent arborizing principally within its internal segment. (Supported by MRC and FRSQ).

436.3 THE TOPOGRAPHICAL EFFECTS OF NOMIFENSINE ON STRIATAL DOPAMINE AS MEASURED BY IN VIVO VOLTAMMETRY. G.E. Glynn* and B.K. Yamamoto (SPON: B. Donzanti). Dept. of Pharmacology, Northeastern Ohio Univs. Coll. Med., Rootstown, Ohio 44272.

Nomifensine is a dopamine uptake blocker that has been used to label dopamine uptake sites in the brain (Scatton et al., 1985) with no direct dopamine releasing properties. The purpose of the present study is to further characterize the neurochemical heteogeneity of the striatum by establishing a "functional" topography of dopamine uptake in the rat caudate-putamen. Male Sprague-Dawley rats were anesthetized with urethane and implanted with stearate-modified carbon paste electrodes (200µm). In vivo voltammetric recordings using linear sweep scans from -0.15 to 0.4V and semidifferential signal processing were made at 5 min intervals. Peak currents were measured at 0.15V for dopamine. Nomifensine (10 mg/kg i.p.) was injected after a 30 min stable baseline. Its effects on extracellular dopamine concentration were studied at 6 areas in the striatum comprising 3 rostrocaudal locations each having a medial and lateral component: anterodorsal medial (ADM) (1.2;1.5;-5.0), anterodorsal lateral (ADL) (1.2;3.5;-4.5), mid-dorsomedial (MDM) (0.2;1.9;-4.5), mid-dorsolateral (MDL) (0.2;3.5;-4.5), posterodorsal medial (PDM) (-0.8;2.7;-4.5), and posteroventral lateral (PVL) (-0.8;4.8;-7.2). Electrode placement was verified by expelling the carbon paste with a 5 V potential. Data were calculated as a percent change from baseline ±SEM.

Minutes	ADL	ADM	MDL	MDM	PVL	PD
60	58±11	58±13	-5± 8	-5±10	21± 9	5± 8
120	135±33	91±28	37±15	5±10	50±16	33±13
180	186±41	97±37	77±19	10±11	62±20	32±17
240	207±49	91±30	82±13	-3±11	71±27	30±19

The results show a definite mediolateral and rostrocaudal gradient in the effects of nomifensine on striatal extracellular dopamine ($p<0.001$). This may in part, be due to the density of dopamine innervation and thus the density of dopamine uptake sites but also suggests a functional topography unrelated to the rostrocaudal gradient of dopamine concentration. This mediolateral difference may correlate with either cortical innervation to the striatum or an unexplained regional difference in dopaminergic function.

436.4 REGIONAL DOPAMINE RELEASE IN RAT STRIATUM MEASURED BY IN VIVO MICRODIALYSIS. B.K. Yamamoto. Dept. of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

We have previously reported a topography of dopamine release in rat striatum using in vivo voltammetry (Neurosci. Abstr., 1986). The present study, using in vivo brain microdialysis, confirms and extends our previous findings that striatal dopamine release and metabolism is topographically organized into subregions. Male Sprague-Dawley rats were anesthetized with urethane and implanted with 400µm dialysis probes (Carnegie Medicin, BAS) having a 2 mm exposed dialysis membrane tip. Probes were implanted into either the anterodorsomedial (ADM) (1.2;1.5;-5.0), anterodorsal lateral (ADL) (1.2;3.5;-4.5), or posterolateral caudate-putamen (PL) (-0.8;4.8;-7.2). The probes were perfused with a modified Ringer's solution at a rate of 2µl/min. The dialysates were collected at 20 min intervals and analyzed for dopamine and DOPAC content by HPLC with electrochemical detection. After a 1 hr stable baseline period, rats were injected i.p. with the D2 antagonist (-)sulpiride (25 mg/kg) or d-amphetamine (2.5 mg/kg) and samples collected for a 4 hr period. All data for dopamine release are expressed as percent of baseline ± SEM (n=4/group).

	Minutes				
	0	60	120	180	240
(-)Sulpiride					
ADL	102± 2	176± 9	203±19*	209±36*	154±21
ADM	100±10	143±16	149±15	142± 6	122±12
PV	103± 8	182±11*	206±15*	211±22*	182±17*
d-Amphetamine					
ADL	100± 5	733±62*	350±33*	250±21*	141±18*
ADM	103± 8	730±54*	308±42*	185+16*	115+21
PV	100± 2	450±30	150±17	87± 9	87±16

*Significantly different from other areas, $p<0.05$.

These results show a medial-to-lateral gradient of dopamine release in response to the D2 antagonist, sulpiride but no rostral-caudal distribution. In contrast, dopamine release in response to d-amphetamine showed a rostral-caudal gradient but no medial-to-lateral effects. Therefore, dopamine release maybe regulated by an interaction between the receptor subpopulation and the degree of dopamine innervation to particular subregions.

436.5 EVIDENCE FOR A HIGH PERCENTAGE OF DOPAMINERGIC, CROSSED MESOSTRIATAL PROJECTIONS IN RABBIT. P.M. Rice*, S-P. Onn, T.W.Berger. (SFON: R. Jennings) Dept. of Behavioral Neuroscience, Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA. 15260.

The mesostriatal projection has been studied extensively, and is considered to be primarily ipsilateral in rats. Only 0.5-2.0% of substantia nigra and ventral tegmental area (SN-VTA) neurons which project to the striatum are located contralaterally. We have studied the mesostriatal projection system in the rabbit using retrograde tracing techniques, and have found a much more substantial contralateral projection.

All animals were adult New Zealand white rabbits. Either wheat germ agglutinin-conjugated HRP (1%, 0.04-0.06 ul) or the fluorescent dye primuline (14%, 0.8 ul) was injected unilaterally into the caudate, and brain tissue was processed, respectively, with TMB histochemistry at 24 hrs or general histology at 48 hrs post-injection. Labelled neurons throughout both sides of SN-VTA complex were scored using dark field microscopy for TMB-reacted HRP, and epi-illumination flourescence for primuline; retrogradely transported primuline appears as yellow granules in the cytoplasm.

Consistent with previous studies, labelled neuronal somata in the contralateral SN and VTA were considerably fewer in number than seen ipsilaterally. In addition, labelled cells appeared to be located at sites corresponding to the center of clusters of ipsilaterally labelled neurons. However, the percentage of contralaterally labelled neurons was found to be 10-16%, significantly higher than reported previously for rats. We further examined the dopaminergic (DA) nature of these crossed projections by combining peroxidase-antiperoxidase or FITC immuncytochemistry for tyrosine hydroxylase with the above retrograde tracers. A combined method for visualizing TMB-DAB cobalt-stablized HRP tracers simultaneously with PAP-labelled immunoreactive markers was developed. Instead of PAP chromogen-DAB, α-naphthol/pyronin was used in conjuction with TMB-DAB stablized reaction products as the immunocytochemical markers. The α-naphthol/pyronin chromogen produces a red/purple precipitate at immunoreactive sites, and thus, has a different color spectrum from the brownish appearance of DAB stablized TMB. This facilitates the identification of double labelling and reduces interference between the two labels. Preliminary data have shown a higher percentage of non-DA projecting neurons both ipsilaterally and contralaterally than previously reported. Supported by NS19608.

436.6 **ULTRASTRUCTURE AND SYNAPTIC CONTACTS OF ELECTROPHYSIOLOGICALLY IDENTIFIED NIGRAL DOPAMINERGIC AND NON-DOPAMINERGIC NEURONS INTRACELLULARLY LABELED WITH HRP** J.M. Tepper, S.F. Sawyer* and P.M. Groves, Dept. Psychiatry, UCSD, La Jolla, CA 92093

Significant differences between the electrophysiological properties of dopaminergic (DA) neurons *in vitro* and *in vivo* suggest that afferents may play a major role in regulating the activity of DA neurons. The aim of the present study was to characterize and compare the morphology and distribution of synapses on electrophysiologically identified substantia nigra pars compacta DA and pars reticulata non-DA neurons, as a prelude to determining the sources of these afferents in conjunction with their electrophysiological effects on identified nigral neurons. Intracellular recordings were obtained from substantia nigra neurons in urethane anesthetized rats with micropipettes filled with 5% HRP in 0.5M KCH_3SO_4. Neurons were identified as DA or non-DA by several well-established electrophysiological criteria including parameters of spontaneous activity and antidromic activation, and were intracellularly labeled with HRP. Vibratome sections of aldehyde-fixed tissue were reacted using 3'-3-diaminobenzidine and embedded in Spurr's medium. The electrophysiological identification and light microscopic morphology of these neurons have been reported previously (Tepper et al., Neurosci. Abstr. 12:1542, 1986), and served as a guide for the ultrastructural analysis of synaptic contacts made onto specific regions of 8 DA and 3 non-DA neurons.

Preliminary observations were based on analysis of the soma, proximal (up to 50 μm from the soma) and intermediate dendrites (50-250 μm from soma) of 2 DA and 1 non-DA neuron, and indicate that DA neurons possess centrally located nuclei, with non-invaginated nuclear envelopes. The cytoplasm was richly endowed with rough endoplasmic reticulum, and a variety of other organelles. DA neurons and proximal dendrites in pars compacta were often closely apposed for several microns by stretches of unlabeled dendrites similar in morphology to labeled dendrites, with no intervening glial membranes. Longitudinally sectioned DA processes were less heavily innervated than homologous regions of similarly sectioned non-DA cell bodies and dendrites in pars reticulata, but both the cell bodies and dendrites of DA neurons received a number of isolated synaptic contacts, whereas the more numerous synapses onto non-DA processes often occurred in groups of 4 or more adjacent boutons lining the dendrite. A large proportion of the synapses onto DA neurons were asymmetrical, comprising at least 2 different classes; small round vesicle synapses with moderate synaptic clefts and prominent postsynaptic specializations, and slightly larger round or pleomorphic vesicle-containing boutons often also containing a few large dense core vesicles that formed very wide synaptic clefts with very dense postsynaptic specializations. In contrast, the vast majority of synapses onto non-DA neurons contained small or large pleomorphic vesicles, and formed symmetric membrane specializations characterized by a narrower synaptic cleft and a thin postsynaptic specialization. This research was supported by grants #DA-02854, RSDA #DA00079 from NIDA and the Office of Naval Research contract #N0001485K0699 to P.M.G.

436.7 NIGROSTRIATAL DOPAMINERGIC ACTIVITY DISSOCIATES EXPRESSION OF DYNORPHIN IN STRIATAL PATCH AND MATRIX NEURONS. C.R. Gerfen, J.F.McGinty, W.S. Young III and L.M. Ibsen*.
Lab Cell Biology, NIMH, Bethesda, MD 20892 and Dept of Anatomy, East Carolina U. School of Medicine, Greenville, NC 27858.

The peptidergic striatonigral systems originate from and the mesostriatal dopamine (DA) systems project to either patch or matrix neurons in a compartmentalized manner (Gerfen, Nature 311:461, '84 and J. Neuroscience, in press). Repeated administration of the DA agonist, apomorphine, (0.5-5 mg/kg, s.c. twice daily) results in a time- and dose-dependent increase in dynorphin and substance P but not enkephalin immunoreactivity in striatal patch, but not in matrix, neurons (Li et al, 1986 and in press). In contrast, destruction of the mesostriatal DA pathway by 6-hydroxydopamine (6-OHDA) increases enkephalin and depresses substance P peptide and mRNA levels throughout the striatum (Young et al PNAS 83:9827, '86).

To determine the functional relationships between the compartmentalized striatonigral and mesostriatal systems, we have used *in situ* hybridization histochemistry to assay the changes in peptide message expression in striatonigral neurons following subchronic apomorphine and/or nigrostriatal 6-OHDA lesions. In normal rats, an ^{35}S-labeled 48 base oligonucleotide probe complementary to the message for dynorphin was used for in situ hybridization combined with fluorescent retrograde dye transport to show that dynorphin is evenly expressed in patch and matrix striatonigral neurons. In rats treated subchronically with apomorphine (5 mg/kg, s.c. twice daily x 9 days) and perfused transcardially with periodate-lysine-paraformaldehyde fixative, the level of dynorphin mRNA in striatal patch, but not in matrix, neurons was increased. The increase in dynorphin message per neuron was positively correlated with upregulation of immunocytochemically identified dynorphin A in patch neurons. Patch neurons with dynorphin upregulation were distributed primarily in the medial and ventral striatum. In additional studies, rats which had received intrastriatal 6-OHDA injections (2μg/μl) as neonates were treated subchronically with apomorphine as adults. In these rats, an enhanced upregulation of dynorphin message levels occurred in areas denervated of dopaminergic afferents, whether in patch or in matrix compartments. These data complement those of Li et al (this volume) which demonstrate by Northern blot analysis a significant increase in striatal dynorphin message levels after a similar apomorphine regimen. Together these studies suggest that dynorphin expression is differentially regulated by dopamine receptors on striatonigral patch versus matrix neurons.
Supported in part by DA 03982 (JFM).

436.8 EVIDENCE FOR A MONOSYNAPTIC RELATIONSHIP BETWEEN DOPAMINERGIC TERMINALS AND CHOLINERGIC NEURONS IN THE RAT STRIATUM: A DUAL-LABELING IMMUNOCYTOCHEMICAL STUDY. H.T. Chang, Department of Anatomy and Neurobiology, The University of Tennessee, Memphis, College of Medicine, The Health Science Center, Memphis, TN 38163.

The synaptic relationship between cholinergic neurons and dopaminergic axons in the rat striatum was examined by a dual-labeling immunocytochemical method. Cholinergic neurons were identified by their immunoreactivity for choline acetyltransferase (ChAT), and dopaminergic axon terminals were identified by their positive immunoreactivity for tyrosine hydroxylase (TH). Monoclonal rat anti-ChAT and monoclonal mouse anti-ChAT antibodies were kindly provided by F. Eckenstein, and G.D. Crawford and P.M. Salvaterra, respectively. Rabbit anti-TH antibodies were purchased from Eugentech. In order to simultaneously visualize and to distinguish both antigens within the same tissue sections at either light or electron microscopic levels, dual-labeling immunocytochemistry procedures were employed in which one of the antigens was labeled by HRP-DAB reaction products while the other was labeled with silver-intensified colloidal gold particles or silver-intensified HRP-DAB reaction products.

At the light microscopic level, large cholinergic neurons were found in both dorsal and ventral striatum. The long and sparsely branched dendrites of these cholinergic neurons were frequently cut at the surfaces of the 50 μm thick Vibratome sections. The surrounding striatal neuropil was densely innervated by TH-positive axon terminals, some of which appeared to contact the cholinergic dendrites. Since detergent was not used in our material, TH-positive axons and terminals were observed only at the surfaces (5 to 6 μm in depth) of the Vibratome sections. Thus, electron microscopic examinations were made only on thin sections taken from the surfaces of the Vibratome sections. Cholinergic neuronal elements labeled by silver-intensified HRP-DAB reaction products were characterized by dark punctate silver particles on top of amorphous DAB reaction products, whereas TH-positive fibers and terminals were labeled by amorphous DAB reaction products only. Numerous TH-positive fibers and terminals were observed to form synapses with unlabeled, and presumably non-cholinergic striatal neurons and dendrites. Identification of synapses between differentially labeled profiles was difficult because synaptic specializations were obscured by reaction products. However, a small number of close appositions, highly suggestive of synapses, were found between TH-positive terminals and ChAT-positive dendrites. This result suggests that dopaminergic axon terminals can have monosynaptic influence on cholinergic interneurons in the rat striatum.

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436.9 THREE-DIMENSIONAL DISTRIBUTION OF CHOLINERGIC PERIKARYA IN THE NEOSTRIATUM OF ADULT CATS. M. Martone*, P. M. Groves, S. J. Young* and D. M. Armstrong (SPON: R. Livingston). Depts. of Neurosciences and Psychiatry, UCSD School of Medicine, San Diego, CA 92093.

In a previous study using three-dimensional computer-assisted reconstructions, we demonstrated that enkephalin-rich patches in the neostriatum of the adult cat are distributed in a highly organized pattern consisting of large finger-like structures radiating diagonally from the ventricular edge across the width of the caudate nucleus. These fingers are connected via numerous smaller crossbridges to give the appearance of a lattice-like pattern of enkephalin immunoreactivity within the caudate nucleus.

In the present study, the 3-dimensional distribution of choline acetyl transferase (ChAT) containing perikarya in the cat neostriatum and their relationship to the enkephalin patches were investigated. Serial 50 um coronal sections were cut through the head and body of the caudate nucleus and stained alternately with antibodies directed against leu-enkephalin (Immunonuclear) or ChAT (generously provided by Drs. L. Hersh and G. Bruce, Univ. of Texas Health Science Center, Dallas, TX). The location of individual ChAT-positive perikarya and the outlines of the enkephalin patches were digitized from camera lucida drawings and entered into an IBM-PC computer. Sections were aligned using the outline of the caudate and major blood vessels for registration.

ChAt-positive perikarya were distributed throughout the neostriatum. As reported elsewhere, the majority of these neurons lie outside of the enkephalin patches. Preliminary observations of limited reconstructions suggest a pattern in the the distribution of these cells similar to that revealed from three-dimensional reconstructions of enkephalin patches. Specifically, clusters of ChAT-positive perikarya appear to form diagonal striations which emanate from the ventricular surface and rings surrounding areas containing few labelled perikarya. The relation of the three-dimensional organization of cholinergic neurons to that of the enkephalin network may provide a framework for understanding information processing within the neostriatum.

This work was supported by the Office of Naval Research ONR N000014-85-K-0699, NIH AG05344 and NIDA 00079 and 02854.

436.10 THE DISTRIBUTION OF GABA ANTIBODY STAINED CELLS PROJECTING FROM THE SUBSTANTIA NIGRA TO THE SUPERIOR COLLICULUS OF THE CAT. A. S. Ficalora*, W. S. Davis*, and R. R. Mize (SPON: K. U. Malik). Dept. of Anatomy and Neurobiology, University of Tennessee, Memphis, TN, 38163.

The projection from the substantia nigra (SN) to the superior colliculus (SC) is thought to be inhibitory and to utilize the neurotransmitter gamma aminobutyric acid (GABA). We have used a double-label technique to study this projection in cat. Specifically, we have asked 1) whether all SN cells projecting to SC are GABAergic; and 2) whether they are selectively distributed within SN. A retrograde tracer, ^{3}H-n-acetylated wheat germ agglutinin (WGA), was injected into SC in order to autoradiographically label the projection neurons from SN. An antibody against BSA-conjugated GABA was used to localize the transmitter in SN.

Virtually all retrogradely labeled cells in SN were also stained by the GABA antiserum, indicating that all SN cells projecting to SC contain GABA. These cells were selectively distributed within SN. Few cells in pars compacta (SNC) were labeled by the GABA antibody and none by ^{3}H-WGA. By contrast, many cells in both pars lateralis (SNL) and pars reticulata (SNR) were double-labeled. The distribution and morphology of these double labeled cells suggests that the two subdivisions form separate projections to SC. A small WGA injection confined to the intermediate layers of SC produced two distinct clusters of labeled cells, one in SNL and the other in SNR. A medium sized injection centered in the intermediate gray layer labeled many more cells, but separate populations were still apparent. A large injection centered in the deepest layers and invading the periaqueductal gray also produced a separate cluster of labeled cells in each subdivision. Since nigrotectal cells were segregated into SNL and SNR regardless of injection size, each subdivision can be said to send a separate, overlapping projection to SC. The morphology of the nigrotectal cells also varied between the two subdivisions, providing further evidence that SNL and SNR contribute separate projections to SC. Nigrotectal cells in SNL were medium to large size (mean average diameter = 30.8 μm). Many of these were large, stellate shaped neurons which were stained intensely by the GABA antiserum. The nigrotectal cells in SNR were smaller (mean average diameter = 26.5 μm) and had varied morphologies. Double labeled cells were also found in the zona incerta (ZI). ZI cells projecting to SC were lightly stained by the GABA antibody. Many had fusiform cell bodies and dendrites oriented in the mediolateral plane.

This study demonstrates directly that virtually all nigrotectal neurons in cat contain the neurotransmitter GABA and are likely to have an inhibitory action on the superior colliculus, as previously demonstrated in other species. SNL, SNR, and ZI each form separate projections to SC. The nigrotectal pathway in cat is therefore more complex than is sometimes recognized. Supported by USPHS grant EY-02973-07.

436.11 HODOLOGICAL STUDY OF GABAERGIC NEURONS IN THE NEOSTRIATUM OF THE RAT. Lorraine Dubé* and Laurent Descarries (SPON: T.A. Reader). Département d'anatomie* and Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, Montréal, Québec, Canada H3C 3J7.

GABA-immunohistochemistry, retrograde axonal transport of HRP-WGA, anterograde degeneration and single section Golgi impregnation were combined to investigate the distribution of cortical and thalamic afferents upon neostriatal GABA-containing neurons projecting to the ipsilateral substantia nigra (SN) in the rat. The HRP-WGA injection sites extended throughout the lateral zona reticulata except for its rostral tip; the cortical and thalamic lesions involved layers V and VI of the somatosensory cortex and the caudal two thirds of the parafascicular nucleus, respectively. Among neostriatal GABA-immunoreactive nerve cell bodies which were retrogradely labeled from the SN, four categories could be distinguished on the basis of size, morphological features and GABA positive input. The majority displayed the characteristics of medium size spiny neurons, i.e. a round, centrally located nucleus surrounded by a thin rim of cytoplasm. In single thin sections, these neurons usually showed 5 to 8 GABA-positive terminals in asymmetrical synaptic contact with their perikaryon, and 1 or 2 per proximal dendritic profile. Other medium size neurons had an elongated cell body containing a smooth ovoid nucleus, and were abutted by 1 or 2 GABA synaptic terminals per section on their perikaryon but 5 to 8 on their proximal dendrites. The third type of medium size neurons exhibited a notched nucleus and these were poorly GABA innervated on their proximal dendrites as well as perikaryon. Finally, a few large striatonigral type II neurons of Bolam et al. '81 did show GABA-immunoreactivity. The SN projecting medium size GABA somata of the three categories were aggregated in clusters among small groups of medium size GABA-containing neurons unlabelled from the SN. Globally, the clustered SN projecting GABA neurons received a denser GABA input than their non retrogradely labeled congeneers. Thus far, all GABA-immunoreactive striatonigral neurons found to be Golgi impregnated fell in the medium size spiny category. Most of these cells were characteristically very densely spinous. They were contacted by degenerating asymmetrical axospinous synaptic boutons of somato-sensory cortical origin. Some others, more abundant in ventral neostriatal sectors, appeared less densely spinous and received degenerating asymmetrical axodendritic synaptic terminals from the parafascicular nucleus of the thalamus. These data indicate that various types of medium size GABA-containing neostriatal neurons project to SN in the rat. This transmitter should be directly implicated in cortico- and thalamo-striatonigral sensorimotor integration since terminals of either origin were demonstrated on two subtypes of the predominant category of GABA striatonigral neurons.(Supported by FRSQ and CRM).

436.12 PARVALBUMIN IS PRESENT IN GABA CONTAINING INTERNEURONS OF THE RAT NEOSTRIATUM. R. L. Cowan , C. W. Wilson and P. C. Emson. Department of Anatomy and Neurobiology, Univ. of Tennessee School of Medicine, 875 Monroe Ave, Memphis TN 38163 USA and Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, UK.

Neurons containing parvalbumin, calcium binding protein, and GABA were immunocytochemically identified in serial semithin sections of the rat neostriatum. Rats were perfused intracardially with a solution of 3% paraformaldehyde and 1% glutaraldehyde. The brains were removed from the animals and were stored overnight in excess fixative. Large blocks of the brains were dehydrated in alcohols and then embedded in polyethylene glycol. A rotary microtome with a metal knife was used to cut 4 um semithin sections from the polyethylene glycol block. The sections were maintained in serial order during all subsequent processing. The sections were treated with a solution of 1% sodium borohydride and placed in a solution of 2% normal serum in KPBS for 4 hours at room temperature. Following the blocking procedure, the sections were incubated for 48 hr in primary antibody solutions of anti-parvalbumin, anti-GABA or anti-calcium binding protein (CaBP/Calbindin) at dilutions of 1:1000-1:2500. The GABA antisera was obtained commercially. The bound antisera were visualized using the avidin-biotin-peroxidase method with diaminobenzidine as the chromogen.

Colocalization of parvalbumin and GABA was studied using adjacent sections stained with anti-GABA and anti-parvalbumin. The distribution of parvalbumin neurons was also compared to that of cells positive for calcium binding protein. CaBP is known to be localized preferentially in neurons of the neostriatal matrix. Parvalbumin containing neurons were found to be present in both CaBP rich and CaBP poor areas of the neostriatum. At least some parvalbumin containing neostriatal neurons also contained GABA. These cells corresponded to the class of larger, more strongly GABA positive neostriatal interneurons, rather than the GABAergic neostriatal projection neurons.

In neocortex and hippocampus, parvalbumin has been associated with rapidly firing interneurons that form axosomatic synapses on projection neurons. Rapidly firing interneurons are occasionally observed in single unit recording studies of neostriatal neurons. The present findings suggest that these neurons may also contain the calcium binding protein parvalbumin.

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436.13 THE EXPRESSION OF SOMATOSTATIN AND SUBSTANCE P IMMUNOREACTIVITY IN PRIMARY CULTURES OF RAT NEOSTRIATUM. E. Galarraga*, D.J. Surmeier, and S.T. Kitai. Dept. of Anatomy nd Neurobiology, College of Medicine, University of Tennessee, Memphis, The Health Science Center, Memphis, TN 38163.

In vivo, neostriatal neurons exhibit somatostatin (SOM) and substance P (SP) immunoreactivity, SOM-immunoreactive neurons are interneurons and constitute a small percentage of the striatal population. On the other hand, SP-immunoreactive neurons contribute to the striatonigral pathway and constitute a large percentage of the striatal population. In the present study we investigated the development of neurons expressing SOM and SP immunoreactivity in monolayer neostriatal cultures prepared from embryonic rat striatum as previously described (Kitai, et.al., Neurosci. Abstr. 12., 653, 1986,).

Studies were performed on neurons in culture 4 to 27 days. For immunocytochemistry, cultures were fixed with 4% paraformaldehyde, 0.2% glutaraldehyde solution. Following treatment with 0.25% triton X-100, cultures were incubated with either SOM or SP antiserum (dilution 1:400 in sodium PBS with 2% normal goat serum for SOM; 1:100 in sodium PBS with 2% normal rabbit serum for SP) for 3 hrs at 4°C. After rinsing, the tissue was incubated for 1 hr. with biotinylated anti-rabbit IgG for SOM and anti-rat IgG for SP (1:100 in sodium PBS with 0.25% triton X-100 and the respective normal serum). Immunoreactive neurons were localized with the biotin-avidin procedure. The HRP-antibody complex was visualized with the diaminobenzidine-glucose oxidase technique.

Specific staining for SOM and SP was seen in all ages studied. Immunoreactivity (IR) was limited mainly to somata and, in the case of SOM, to some proximal processes. The size and shape of immunoreactive cells varied; most of these cells were bipolar (80% SOM-IR, 88% SP-IR), but tripolar (17.5% SOM-IR, 8% SP-IR) and multipolar (2.5% SOM-IR, 4% SP-IR) cells were also stained. Our results indicate that the proportion of SOM-IR cells increased with culture age; from 19% (4 DIV) cultures to 30% (25 DIV). In contrast, the proportion of SP-IR neurons decreased from 20% (6 DIV) to approximately 13% (27 DIV). No evidence for induction or delayed-expression of either phenotype was observed. As indicated by the changes in proportion, SP-IR neurons decrease in number more rapidly than SOM-IR neurons. This suggests long term survival in pure striatal culture could be favored for interneurons.

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436.14 STRIATONIGRAL PROJECTION NEURONS: A RETROGRADE LABELING STUDY OF THE RELATIVE NUMBERS THAT CONTAIN SUBSTANCE P OR ENKEPHALIN. K.D. Anderson and A. Reiner. Dept. of Anatomy & Cell Biology, Univ. of Michigan, Ann Arbor, MI 48109.

Two largely separate populations of neuropeptide-containing striatonigral projection neurons have been distinguished in pigeons, one population whose neurons contain substance P (SP) and dynorphin (DYN) and a second population whose neurons contain enkephalin (ENK) (Reiner, Brain Res., '86, Soc. Neurosci. Abs., '86). Since GABA has also been found in many striatonigral projection neurons, it has not been clear whether all striatonigral projection neurons contain GABA as well as either SP/DYN or ENK, or whether many striatonigral projection neurons contain GABA but neither SP/DYN nor ENK. To answer this question, we determined the percentage of striatonigral projection neurons that contain SP/DYN or ENK, using retrograde labeling with the fluorescent dye fluorogold in combination with immunofluorescence labeling for these neuropeptides.

Pigeons received intranigral injections of fluorogold, were colchicine-treated three days later and perfused transcardially with fixative 36 hours subsequently. Adjacent series of sections through the basal ganglia were labeled with antisera against SP (since SP and DYN are almost entirely found in the same striatal neurons, we did not label neurons for DYN) or ENK. The tissue was viewed using fluorescence microscopy and, by switching back and forth between the appropriate sets of excitation/emission filters, we could determine if individual fluorogold+ retrogradely-labeled neurons were also immunolabeled. We found that 80-85% of the fluorogold+ striatal neurons were SP+, while only 5-10% were ENK+.

Thus, the majority of the striatonigral projection neurons contain SP, while a much smaller population contain ENK. Only a small percentage of the striatonigral projection neurons (5-15%) were not observed to contain SP or ENK. These neurons might have contained amounts of SP or ENK that were subthreshold for detection, or they might contain some other neurotransmitter, perhaps GABA. However, since the GABAergic striatonigral projection in birds is substantial (Reiner, Soc. Neurosci. Abs., '86), GABA must also be present in SP+ (and presumably also ENK+) striatonigral projection neurons. This conclusion is directly supported by our immunohistochemical double-labeling studies examining SP-GABA co-occurrence (Reiner and Anderson, Soc. Neurosci. Abs., '87). Taken together, the present results and our immunohistochemical double-labeling data indicate that the majority of the striatonigral projection neurons contain SP, DYN and GABA and a smaller population of neurons contain ENK and presumably also GABA. Supported by NS-19620 (A.R.).

436.15 THE CO-OCCURRENCE OF SUBSTANCE P AND GABA IN STRIATAL PROJECTION NEURONS OF THE BASAL GANGLIA. A. Reiner and K.D. Anderson. Dept. of Anatomy & Cell Biology, Univ. of Michigan, Ann Arbor, MI 48109.

Substance P (SP) is found in both striatopallidal and striatonigral projection neurons. GABA is also found in both of these types of striatal neurons, but it has been unclear if SP and GABA are found in separate populations of neurons, or whether the SP+ neurons also contain GABA. To examine this issue, we have used simultaneous immunofluorescence double-labeling techniques (Reiner, Brain Res., '86). These studies were carried out in turtles, pigeons and rats to assess the extent to which any observed SP-GABA co-occurrence is a phylogenetically conservative feature of basal ganglia organization.

Two approaches were used. The first was to examine the extent of SP-GABA co-occurrence in striatal perikarya in sections from colchicine-treated animals. A rabbit antiserum against GABA (from Drs. J. Hildebrand and T. Kingan) and a rat monoclonal antibody against SP were used. We found that many of the SP+ perikarya throughout the striatum in turtles, pigeons and rats were also labeled for GABA. This was particularly striking in rats, in which at least 90% of the SP+ striatal perikarya were also GABA+. The second approach was to examine fibers and terminals in the substantia nigra and globus pallidus (or the entopeduncular nucleus in the case of rats) for the co-occurrence of SP and glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA (using sheep anti-GAD from Dr. I.J. Kopin). Since nearly all of the SP+ fibers and terminals in these regions arise from striatal neurons, the extent of SP co-occurrence with GAD in these regions reflects the extent of SP-GABA co-occurrence in striatal projection neurons. The results indicated the presence of GAD in the vast majority of the SP+ fibers and terminals in these striatal target areas. However, in the studies both of striatal perikarya and of striatal target areas, many GABAergic perikarya, fibers and terminals were observed that were *not* SP+.

These studies demonstrate that the majority of the SP+ striatal projection neurons also contain GABA and that this is an evolutionarily conserved feature of basal ganglia organization. The vast majority of these neurons also must contain dynorphin, since SP and dynorphin are nearly always found in the same striatal neurons (Reiner, Brain Res., '86; Besson et al., Soc. Neurosci. Abs., '86). The present results also show that many GABAergic striatal projection neurons do not contain SP. It seems likely that many of these GABAergic neurons contain enkephalin (ENK). Although it is possible that some GABAergic striatal projection neurons contain neither SP nor ENK, other results of ours (Anderson and Reiner, Soc. Neurosci. Abs., '87) suggest that, at least for striatonigral projection neurons in pigeons, this could be, at most, only a minor population. Supported by NS-19620 (A.R.).

436.16 NEUROPEPTIDE-LIKE IMMUNOREACTIVITY IN THE SUBSTANTIA NIGRA AND ENTOPEDUNCULAR NUCLEUS IN THE NORTH AMERICAN OPOSSUM. J.C.Hazlett, R.H.Ho and G.F.Martin. Department of Anatomy and Cell Biology, Wayne State University, Detroit, Michigan 48201 and Department of Anatomy, The Ohio State University, Columbus, Ohio 43210.

The major output centers of the basal ganglia, the substantia nigra (SN) and entopeduncular nucleus (EPN), contain peptidergic neurons and receive peptidergic projections. This information is based on studies in the rat, cat and monkey, however, and to the best of our knowledge, peptides have not been described in the basal ganglia of marsupials. Because of that and our continued interest in the opossum basal ganglia, we have used the PAP method to identify cholecystokinin (CCK), methionine-enkephalin (ENK), somatostatin (SOM) and substance P (SP)-like immunoreactive structures in the EPN and SN of the adult opossum. A moderate number of neuronal somata containing CCK-like immunoreactivity was observed throughout pars compacta, pars lateralis and the caudal half of the pars reticulata of the SN. Small numbers of CCK positive axons were present in the pars compacta and pars lateralis. Such axons were also found within the retrorubral nucleus (RRN), the ventral tegmental area (VTA) and the EPN. A dense ENK fiber plexus was present throughout the SN, RRN and VTA. ENK immunoreactive fibers were moderately dense in the EPN, but particularly dense in the globus pallidus. A small number of SOM-like immunoreactive perikarya was present in pars lateralis, the pars compacta and the caudal two-thirds of the pars reticulata of the SN. A moderate number of SOM-like immunoreactive neurons was also seen in the RRN and EPN. SP-like immunoreactivity was present in an extensive fiber plexus within the SN and EPN. The density of these fibers appeared greatest in the medial pars reticulata at mid-nigral levels. A few somata showing SP-like immunoreactivity were present in the VTA. In summary, the general distribution of CCK, ENK and SP-like immunoreactivity in the opossum's substantia nigra and entopeduncular nucleus resembles that described in other species.

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436.17 NEURONAL MIGRATION IN PRIMARY MONOLAYER CULTURES OF RAT NEOSTRIATUM. D.J. Surmeier, P. Akins and S.T. Kitai, Dept. of Anatomy and Neurobiology, U. of Tennessee, Memphis, TN 38163.

Neuronal migration is an important developmental process. The factors governing it are only beginning to be understood. The primary tissue culture model of the developing brain has provided some insights into this process. Hatten et al. (J. Cell Biol., 98:193, 1984) found that granule cell migration in cerebellar cultures was dependent upon specific glial-neuronal contacts: this migration also appears to follow a time course similar to that expected in vivo (Trenkner et al., J. Neurosci., 4:2850, 1984). Although the adult neostriatum lacks the laminar cytoarchitecture that characterizes the cerebellum, it does possess inhomogeneities in cellular phenotype that suggest neuronal migration patterns and cell-specific interactions could play an important role in determining its organization. Therefore, a quantitative analysis of neuronal migration was attempted using primary dissociated cultures of embryonic neostriatum.

Striata from E17 rat embryos were aseptically dissected into calcium, magnesium free Hank's balanced salts buffered with 10 mM HEPES. Mechanically dissociated cells were plated onto glial feeder layers or poly-l-lysine at 0.5-1.0 X 10^6 cells/35 mm petri dish. The growth dishes were branded with a grid composed of 300 um squares that allowed the identification of particular neurons at successive intervals. Neuronal movement was tracked using still and time-lapse photography from plating to 34 DIV.

Initial experiments have yielded several observations pertinent to the characteristics of striatal migration and the factors governing it. Migratory rates declined steadily with in vitro age, with the primary phase of migration lasting 2-3 weeks. The mean rate of movement of isolated neurons declined from 12 um/hr initially to 3-6 um/hr at two weeks to 1-2 um/hr at 3 weeks in vitro. Mean rates were correlated with neuronal morphology, being greatest for bipolar neurons, smallest for multipolar neurons. Neurons did not appear to migrate in conjunction with any particular glial morphology and somatic translocation was not dependent upon neuro-neuronal contact. Aggregates of neurons formed soon after plating (10-30 minutes) as a consequence of neuronal migration. Aggregates were initially transitory but achieved greater stability over the succeeding days. Substantive neuronal migration between aggregates, however, continued for at least 2 weeks after plating. These results suggest that striatal organization may not be complete until the second postnatal week.

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436.18 TIME OF ORIGIN OF IMMUNOHISTOCHEMICALLY IDENTIFIED INTERNEURONS IN THE RAT STRIATUM. H.C. Fibiger, K. Semba and S.R. Vincent. Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, Vancouver, B.C. V6T 2A1 Canada.

The presence of cholinergic and somatostatin (SOM)-containing neurons has been demonstrated in the striatum. Both neurons are believed to be interneurons, and morphologically correspond to the large and the medium aspiny neurons, respectively. To obtain a better understanding of the organizing principles of the striatum, the development of cholinergic and SOM-containing neurons was studied using combined tritiated thymidine autoradiography and immunohistochemistry. Tritiated thymidine was injected into timed pregnant rats (conception designates day 0), and the brains of their progeny as young adults were processed for immunohistochemistry using monoclonal antibodies to choline acetyltransferase (ChAT) and SOM, followed by autoradiography. The time of final mitosis of the neurochemically identified neurons was determined by analyzing the proportion of neurons double labelled with the antigen and tritium among all immunolabelled neurons, as a function of the day of thymidine injection.

The majority of ChAT-immunoreactive neurons in the striatum became post-mitotic on embryonic (E) day 12-13, although there was a caudal to rostral gradient in cholinergic neurogenesis. At caudal levels of the striatum, the peak of final mitotic activity occurred on E12. At intermediate levels, most ChAT-positive neurons underwent their final mitotic division on E12 and E13. ChAT-positive neurons at the most rostral levels of the striatum became post-mitotic on days E12 to E15. In contrast, the neurogenesis of SOM-immunoreactive neurons occurred after that of the ChAT-positive interneurons, mostly on E15 and E16, irrespective of the level within the striatum. The neurogenesis of both ChAT- and SOM-positive neurons was essentially complete by E17.

The present data indicate that: (1) cholinergic interneurons in the striatum leave the mitotic cycle mostly on E12-13, approximately 3 days earlier than SOM-containing interneurons, and (2) there is a gentle caudal to rostral gradient in cholinergic neurogenesis. The differences in the spatiotemporal pattern of neurogenesis between these two interneurons may be related to differences in their function.

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436.19 PATCH STRIATONIGRAL NEURONS PREFERENTIALLY SURVIVE THE PERIOD OF STRIATAL CELL DEATH G. Fishell & D. van der Kooy, Neurobiology Research Group, Dept. of Ana., University of Toronto, Toronto, Canada M5S 1A8

The adult striatum can be divided into two complementary and phenotypically distinct compartments, the patches and the matrix. Although the compartments are established late in embryogenesis, many phenotypic markers only come to respect the boundaries of the compartments during the early postnatal period. During this same early postnatal period the striatum undergoes significant cell death with estimates of its extent varying from 25 to 50%, depending on the species . We now ask whether this striatal cell death is compartment specific. Neurons of the patch compartment become postmitotic and send axonal projections to the substantia nigra earlier during development than neurons of the matrix compartment By injecting retrograde tracer into the substantia nigra of E18 rat embryos, we were able to specifically label neurons within the striatal patch compartment . The distribution and number of retrogradely labeled patch neurons was then compared at postnatal day (PND) 2 and PND17. The distribution of retrogradely labeled cells was correlated with another patch marker (opiate receptors) in adjacent sections. Opiate receptors were visualized in adjacent sections by incubation in radioactively labeled opiate receptor ligands ([^{3}H] Etorphine or [^{125}I] Dago)

The distribution of embryonically labeled striatonigral neurons exactly matched the distribution of patches of opiate receptors at both PND2 and PND17. Moreover , the number of retrogradely labeled cells within the entire patch compartment over this same period does not change significantly, despite the reported 25% decrease in total striatal neurons.

These observations provide evidence in support of the idea that maintenance of the striatal compartments is intrinsically controlled. Both cell death and increases in the neuropil of striatal cells change the shape and size of the patch compartment during the the first few postnatal weeks. The maintained matching of patch boundaries (as measured by opiate receptor distribution and the distribution of cells retrogradely labeled from an embryonic injection) suggests that a specific set of patch cells and not an extrinsicly determined spatial area delineates compartmental boundaries. Second, it appears that the early striatonigral patch projection cells preferentially survive the period of cell death. This suggests that an early projection to the substantia nigra may be a factor in giving patch cells a competitive advantage.

436.20 PRENATAL AND POSTNATAL ONTOGENY OF THE DOPAMINERGIC INNERVATION OF THE RAT STRIATUM. P.Voorn*, A.Kalsbeek* and H.J.Groenewegen. Dept. Anatomy, Free University (PV, HJG) and Neth. Inst. Brain Research (AK), Amsterdam, the Netherlands.

In the adult rat the striatum has a compartmentalized structure, reflected in the inhomogenous distribution of dopamine (DA). To test the hypothesis that DA plays an organizational role in the development of the striatum, the ontogeny of the DA system was studied in detail with immunocytochemical methods employing antibodies against DA. Rat embryos, foetus, pups and adults were perfusion-fixed with glutaraldehyde on all prenatal days from E11 onward, postnatally on P2, P4, P6, P8, P13, P20, and in adult age. On E13 the first DA cells are detected in the ventral prosencephalon. On E14 two DA cell groups are present in the ventral mesencephalon, and projections of the cells reach the ventrolateral parts of the ganglionic eminence. In the next two days both cell groups and projections increase rapidly in size. On E17 the afferent DA fibers to the striatum become aligned and appear as huge bundles, that are closely associated with the internal capsule. Rostrally, the development of the striatal DA innervation shows a clear ventromedial to dorsolateral gradient, whereas more caudally the DA fibers innervate the striatum from a ventromedial position. The lateral parts of the otherwise compact mesencephalic cell groups consist of loosely arranged cells. These cells start arranging on E17 in a dorsal and ventral group. Just before birth, on E21, the primordia of the substantia nigra pars compacta and pars reticulata can be observed. On E19 several centers with extensive fiber ramifications along the dorsolateral margin of the caudate-putamen represent the first signs of the inhomogenous distribution of DA fibers in the dorsal striatum seen during the next two weeks. In the following pre- and postnatal days also more medially these so-called DA "patches" appear. By the third postnatal week most of these are no longer detectable, and only the most dorsolaterally located "patches", i.e. lying in the region where they first were detected on E19, remain visible in the adult stage. Prenatally, no varicosities can be observed in the DA fibers. The first varicosities appear just after birth. Their number increases rapidly during the first and second postnatal weeks and reaches near adult levels on P20. It is concluded that prenatally the DA fibers are not preferentially located in "patches", but that the inhomogenous distribution is caused by "festerings" of DA fibers in particular regions. It can further be concluded on the basis of the present results and those of thymidine autoradiographic studies that the development of the DA innervation of the striatum and the neurogenesis of this area are very closely related.

437.1 IMMUNOCYTOCHEMICAL ANALYSIS OF CENTRAL OXYTOCIN DISTRIBUTION DURING PREGNANCY AND POSTPARTUM, G.F. Jirikowski*, J.D. Caldwell* and C.A. Pedersen Abt. Anatomie und Zelibiologie, Univ. of Ulm, Postfach 4066, Ulm, F.R.G.; Department of Psychiatry, School of Medicine,University of North Carolina, Chapel Hill, NC 27514

The central distribution of the nonapeptide oxytocin may account for its effects on behaviors as maternal and sexual behavior. The ovarian steroids affecting these behaviors influence oxytocin immunoreactive levels and topography (Soc. Neurosci. Abs. 12:376.15,p.1388 and 184.11,p.673). We have previously demonstrated changes in oxytocin immunoreactive levels during late pregnancy and peripartum (Caldwell et al. Neuroendo. Vol 47 in press). In this study we examined how oxytocin distribution changes during pregnancy and postpartum in mice by examining serial brain sections stained against oxytocin antibody. Animals were killed on day 15 or 18 of pregnancy, at parturition or at day 2, 5 or 30 postpartum. Vibratome sections were incubated over night in anti-oxytocin antisera diluted 1:5000 in PBS containing 0.05% Triton X 100 at 4° C. Immunoprecipitates were visualized with the peroxidase-anti-peroxidase method. Immunostained perikarya were visible in all experimental groups in the magnocellular nuclei as well as in various accessory nuclei.

Unlike cells in the magnocellular nuclei, that showed no changes across pregnancy and peripartum, oxytocinergic neurons in "accessory" nuclei showed varying topography across these states. Oxytocinergic cells in the lateral subcommissural nucleus, preoptic region and the perifornical region were clearly clustered around blood vessels in late pregnancy and postpartum. Cells in these same areas and in the anteroventral zona incerta were scattered and not associated with blood vessels in lactating animals. Whereas these clustered cells sent their projections along blood vessels, scattered cells seen in lactating animals clearly sent projections through the neuropil to central sites. Oxytocinergic brain topography of mothers after weaning was comparable to the topography in ovariectomized control mice. While perivascular oxytocin neurons perhaps supplement the high levels of oxytocin necessary peripartum, centrally projecting oxytocin neurons in lactating animals may influence postpartum behaviors such as maternal behavior.

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437.2 EFFECT OF CASTRATION AND TESTOSTERONE TREATMENT ON SUBSTANCE P LEVELS WITHIN THE VOMERONASAL PATHWAY OF THE MALE GOLDEN HAMSTER. Swann J.M. and Newman S.W. Department of Anatomy and Cell Biology, Univ. of Michigan, Ann Arbor, MI 48109-6005

Male mating behavior in most vertebrate species is dependent on circulating gonadal steroids. While it is clear that steroids regulate mating behavior through action on the CNS the neurochemical mechanisms which mediate steroidal action are poorly understood. Mating behavior in the male golden hamster is regulated by three critical areas of the CNS: the medial nucleus of the amygdala (M), the medial preoptic area (MPOA) and the medial part of the bed nucleus of the stria terminalis (BNSTm). As neurons in these areas actively accumulate gonadal steroids and contain a number of peptide neurotransmitters, we hypothesized that steroidal regulation of mating behavior may occur through changes in neuropeptide levels. To test this hypothesis we compared immunohistochemical levels of substance P in M, the MPOA and the BNST in intact, castrate, and castrate/testosterone-treated hamster.

Male golden hamsters that were castrated for 13 weeks (N=4) castrated for 13 weeks and treated with testosterone (500 ug every other day for 4 weeks, N=4) or gonadally intact (N=3) were injected with colchicine (160-320 mg into the lateral ventricles). Each animal was perfused 48 to 72 hours later with paraformaldehyde fixative containing benzoquinone. Brains were removed, cut into 40 um sections on a freezing microtome and incubated sequentially with a monoclonal antibody to substance P (Accurate Scientific), a bridging antibody and peroxidase-antiperoxidase (PAP) complex. The complex was visualized with DAB enhanced by nickel chloride.

In the intact hamsters neurons and fibers containing substance P-like immunoreactivity (SP-IR) were identified throughout the MPOA, BNST and M. In the BNST the greatest percentage of these neurons and fibers were found in the medial subdivision, with the highest concentration in the dorsal part of the postcommissural BNSTm just medial to the stria terminalis. In the M the highest concentration of neurons containing SP-IR was located in the caudal 2/3 with a dense accumulation of cells and fibers in the caudal-most tip just rostral to the amygdalo-hippocampal area. In the MPOA the highest concentration of neurons containing SP-IR was in the caudo-lateral 1/3, while the highest fiber density occurred in the periventricular and medial regions. SP-IR of fibers and cells in the castrated animals was substantially decreased in the BNSTm and the caudal part of M and to a lesser extent in the MPOA as well, while castrates treated with testosterone had an intensity of SP-IR fibers and cells comparable to and in some cases, greater than that of the intact animals.

These results indicate that substance P levels within M, the MPOA and the BNSTm are regulated by gonadal steroids and suggest that changes in the levels of this peptide may play a role in steroidal regulation of mating behavior in this species.

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437.3 TESTOSTERONE REGULATES THE SUBSTANCE P INNERVATION OF THE MEDIAL NUC. OF THE AMYGDALA. C.W. Malsbury, K. McKay* & K. Hansen*. Dept. Psychology and Div. of Basic Medical Sciences, Memorial U. of Newfoundland, St. John's, Nfld, Canada, A1B 3X9.

The medial nuc. of the amygdala (M) is known to be involved in the control of gonadotrophic hormone release and in the control of testosterone-sensitive behavior (mating, aggression). The most caudal part of M stains very darkly for substance P (SP) in both sexes, but the area of this dense SP innervation is over twice as large in males as in females (Malsbury & McKay, in preparation). This sex difference raised the question of whether testosterone (T) might regulate the SP innervation of M in the adult. Dees & Kozlowski (Neuroendocrinol., 1984, 39:231) reported that the density of SP staining in M was reduced at 10 days post-castration. We have examined this question by measuring the area of dense SP staining in M in adult male rats at 2, 4 and 8 wks post-surgery. Three groups (n=4/group); sham operated (SHAM), castrated (C), and castrated plus T (C+T) were prepared for each survival period. Group C+T animals each received (sc) a 45 mm silastic capsule of T at the time of castration. Brain sections from one animal of each of the three groups were processed simultaneously for immunocytochemistry using the PAP method. The size of the discrete region of dense staining in alternate, 35 micron sections was quantified using an IBM-PC-based area measurement program. The perimeter of the region was traced bilaterally for each coronal section and the sum of the areas was calculated for each animal without knowledge of group membership. The means (SD) of the summed cross-sectional areas for the 2 and 8 wk groups are shown below. By 2 wks, the area of staining was 20% smaller in group C as compared to the matched sham-operated group. By 4 wks, the area was 25% smaller in group C (data not shown), and by 8 wks it was 42% smaller. However, even at 8 wks post-castration, the size of the area was not as small as in normal females (mean = 4.65 sq. mm). T maintained the size of the stained region in castrated males. Thus, the size of a presumed SP terminal field in the most caudal part of M is regulated by T. The gradual decline in its size following castration is interesting, as it parallels the gradual decline in sexual behavior which follows castration.

AREA (SQ. MM) OF SP STAINING IN M

2 WK			8 WK		
SHAM	C	C+T	SHAM	C	C+T
10.72	8.62	10.92	11.64	6.77	10.44
(.56)	(.87)	(.57)	(.53)	(.36)	(.58)

437.4 IS THERE A DIFFERENTIAL TISSUE DISTRIBUTION OF NPY AND NOR-ADRENERGIC NERVES IN THE FEMALE REPRODUCTIVE TRACT? R.E. Papka and H.H. Traurig. Dept. of Anatomy and Neurobiol., Univ. KY, Lexington, KY 40536.

It is becoming established that nerve fibers of many tissues and organs may co-store two (or more) neurotransmitter substances. In some organs, e.g. the gut, it has been demonstrated that nerves chemically coded for a certain transmitter, or combination of transmitters, may innervate different tissues depending on their chemical "signatures". Neuropeptide tyrosine (NPY) and nor-adrenaline (NA) have been demonstrated to co-exist in many peripheral autonomic neurons. We undertook a study to examine the possible differential innervation of tissues in the reproductive tract by nerves that may contain one putative transmitter substance (either NA or NPY) vs. those that may co-store two or more putative transmitter substances (e.g. NPY and NA). Experiments were designed to determine if nerves containing only NPY or only NA innervate myometrial smooth muscle and if fibers innervating vascular smooth muscle co-store NPY and NA. Animals were treated with the noradrenergic neurotoxin 6-hydroxydopamine (6-OHDA) and then the uterine cervix examined immunohistochemically for NPY^+ and tyrosine hydroxylase $(TH)^+$ (noradrenergic) fibers. In control animals NPY^+ fibers were numerous in the myometrium and around blood vessels; TH^+ fibers were few in the myometrium and moderate in number around blood vessels. The numerous myometrial NPY^+ fibers were not affected by 6-OHDA treatment; in contrast NPY^+ fibers were markedly reduced around blood vessels. TH^+ fibers were not evident in the myometrium nor around blood vessels following 6-OHDA treatment. We concluded that most myometrial NPY^+ fibers did not contain NA since they were not sensitive to 6-OHDA; however, perivascular NPY^+ fibers which degenerated after 6-OHDA treatment probably co-store NA. To further examine the question of co-storage, adjacent cryostat sections were stained for NPY and TH and searched for staining overlap in myometrial and perivascular nerves. Good evidence of staining overlap was observed in perivascular nerves but not in myometrial nerves. Thus, there is probably a differential innervation of tissues in the reproductive tract by nerves containing one vs. more than one putative transmitter substance. It is possible that the target tissues provide some of the coding for the transmitters and types of nerves innervating them. (Supported by NIH Grant NS22526).

437.5 STUDIES ON THE INNERVATION OF THE IMMATURE RAT OVARY BY CGRP. J. Calka*, J.K. McDonald, L. Jordan *, M.E. Costa,* S.R. Ojeda. Dept. of Anatomy & Cell Bio., Emory Univ. Sch. Med., Atlanta, GA 30322 and Neuroscience Division, Oregon Regional Primate Research Ctr., Beaverton, OR 97006.

Nerve fibers in the guinea-pig ovary display calcitonin gene-related peptide (CGRP)-like immunoreactivity (Uddman et al. Regul. Peptides 15:1, 1986). The present study investigated; 1) the presence and distribution of CGRP immunoreactive nerve fibers in the immature rat ovary, 2) the route by which these fibers enter the ovary and 3) the potential effects of CGRP on estrogen (E2) and progesterone (P) secretion in vitro.

Female Sprague-Dawley rats (28-30 days of age) were perfused with 4% paraformaldehyde, 0.1M DL-lysine HCl and 0.01M sodium periodate. Additional ovaries were fixed by immersion in Zamboni's fixative. Other animals were subjected to transection of the abdominal vagus nerves (AV), superior ovarian nerves (SON) or plexus nerves (PN), or both the SON and PN at 24 days and sacrificed 6 days later. Ovaries were sectioned and processed for indirect immunofluorescence using antiserum against [Tyr]rat CGRP (23-27) at a dilution of 1:8000 (kindly provided by Dr. C. Sternini). Fluorescein-labeled secondary antiserum was used at 1:100. Preabsorption of the antiserum with 1μM synthetic CGRP blocked all specific staining. In ovaries from intact rats, many CGRP-labeled fibers surrounded blood vessels, although additional fibers coursed through the interstitial tissue. Transection of the AV or SON had no discernible effect on CGRP staining. In contrast, sectioning of the PN or combined SON and PN section eliminated all CGRP-like immunoreactivity in 5 of the 6 ovaries examined for each condition. In one ovary from each of these groups, a small number of labeled fibers were observed.

CGRP, at doses ranging from 10^{-10} to 10^{-6} M, failed to alter E_2 and P secretion from whole ovaries incubated in vitro or from granulosa cells of hypophysectomized, estrogen treated rats in culture. The peptide also failed to affect the E_2 and P response of these cells to FSH.

The results demonstrate that CGRP-immunoreactive fibers enter the ovary via the PN and are distributed predominantly with the vasculature. The function of CGRP in the immature ovary is unknown, but it may be more related to the control of blood flow than to the direct regulation of steroidogenesis.

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437.6 COMBINED RETROGRADE TRACING AND IMMUNOCYTOCHEMICAL STUDIES OF NEURONS IN PARAVERTEBRAL GANGLIA PROJECTING TO THE RAT OVARY. C.M. Klein* and H.W. Burden. Dept. of Anatomy & Cell Biology, East Carolina Univ. Sch. of Med., Greenville, NC 27858.

Substance P (SP)- and vasoactive intestinal peptide (VIP)-immunoreactive nerve fibers have previously been described in the rat ovary (Kannisto et al., Histochemistry,86:25, 1986; Papka et al., Cell and Tissue Res.,242:475, 1985). Using combined retrograde tracing and immunocytochemical techniques we recently reported that 20% of sensory nerve cell bodies in dorsal root ganglia projecting to the ovary contained VIP immunoreactivity and 23% contained SP immunoreactivity (McNeill and Burden, Amer. J. Anat.,in press, 1987). No perikarya in prevertebral ganglia projecting to the ovary were immunoreactive for SP or VIP. We have recently observed that many postganglionic nerve cell bodies innervating the ovary reside in paravertebral gnaglia. In the current study we utilized retrograde tracing with the fluorescent dye True Blue (TB) and immunocytochemistry to determine whether perikarya residing in paravertebral ganglia which project to the ovary contain SP or VIP immunoreactivity. The superior ovarian nerve was sectioned unilaterally and the central end was isolated and immersed for two hours in a 5% solution of TB. Six days later, rats were perfused through the ascending aorta with fixative and paravertebral ganglia from lower thoracic and upper lumbar segmental levels were removed. The tissue was sectioned at 15 um in a cryostat and placed on glass slides, and then processed for SP or VIP immunoreactivity by the indirect immunofluorescence technique. SP- and VIP-immunoreactive fibers and VIP-immunoreactive perikarya were found in paravertebral ganglia. In some instances, VIP-immunoreactive fibers were in close association with perikarya labeled with TB. Some perikarya labeled with TB were also immunoreactive for VIP. This observation indicates that some ovarian VIP-containing nerve fibers originate from paravertebral ganglia. Some VIP-immunoreactive fibers in paravertebral ganglia may be collaterals of primary sensory neurons. The presence of these fibers in close relation to principal ganglion cells projecting to the ovary provides an anatomical substrate for reflex modulation of ovarian function.

437.7 EXPRESSION OF THE CORTICOTROPIN RELEASING HORMONE GENE IN THE HUMAN PLACENTA.

M. Grino*, A.N. Margioris*, D.D. Brandon*, A.J. Markwick* and G.P. Chrousos, (Spon: J.E. Barrett) DEB, NICHD, NIH, Bethesda, MD 20892

Human placenta is a rich source of several hypothalamic and pituitary hormones. Recently, plasma immunoreactive corticotropin releasing hormone (IR-CRH) concentrations were found to rise progressively during pregnancy. After parturition plasma IR-CRH levels decrease rapidly within hours. Human term placenta homogenates contain large amounts of IR-CRH and we have shown that human term placenta fragments secrete IR-CRH in vitro. The bulk of placenta and circulating IR-CRH in pregnancy exhibit the same apparent MW as synthetic human CRH.

To investigate the origin of the high plasma levels of IR-CRH in pregnancy, we first searched for the possible expression of the CRH gene in human term placenta. Placental RNA was extracted by the guanidine thiocyanate-cesium chloride method, separated by agarose gel electrophoresis, blot transferred and hybridized with a radiolabelled synthetic oligonucleotide corresponding to the amino acids 22-37 of the human CRH sequence. In the human placenta RNA lane, a single band of about 1300 nucleotides hybridized with the CRH probe; a single band, of the same size, was found in the lane of poly (A)-RNA enriched extract from rat hypothalami.

We conclude that the CRH gene is expressed in human term placenta. This finding, together with the finding of placental IR-CRH secretion in vitro, further supports the hypothesis that the high concentration of IR-CRH in the plasma of pregnant women is of placental origin. The regulation of placenta CRH is still unknown. We are currently working along these lines.

437.8 IMMUNOCYTOCHEMICAL EVIDENCE FOR A LAMPREY-LIKE GONADOTROPIN-RELEASING HORMONE IN HUMAN BRAIN. E. G. Stopa*, S. A. Sower *, C. N. Svendsen* and J. C. King (SPON:L. Adelman). New England Medical Center and Tufts University School of Medicine, Boston, MA 02111, University of New Hampshire, Durham, N.H.03824, and Brain Tissue Resource Center, McLean Hospital/Harvard Medical School, Belmont, MA 02178.

Gonadotropin-releasing hormone (GnRH) was first isolated from porcine and ovine hypothalamic extracts. Peptides with an identical sequence have also been isolated from the hypothalami of a number of other species of mammals, giving rise to the view that a single form of GnRH [mGnRH] is present in all mammals. The present study was undertaken to test the hypothesis that a non-mammalian form of GnRH might be conserved during evolution and found in human brain. Immunocytochemistry was used to examine human tissues obtained within 6-18 hours postmortem. Two antisera generated to m-GnRH (1076, CRR11B73) and two antisera generated to l-GnRH (1467, 1459) were used in these studies. Antisera generated to l-GnRH (1467, 1459) demonstrated less than 0.01% and 0.02%, cross reactivity, respectively, with m-GnRH. To eliminate the possibility of cross-reactivity in these immunocytochemical studies, the diluted antisera were preincubated with mammalian LHRH at a concentration of 10^{-6}M at 4 ^{0}C overnight. The hypothalamus was fixed by immersion for 24 hours in 5% acrolein in 0.1 M phosphate buffer (pH 7.2). The immunocytochemical procedures used have been described in detail (King, et al., J Clin Endocrinol Metab 60:88,1985). Neuronal cell bodies and fibers, immunoreactive with antisera (1467 and 1459) were not confined to specific nuclei, but were dispersed throughout the preoptic area and basal hypothalamus in all brains examined (2 female and 2 male). These immunoreactive neurons were primarily bipolar and were considerably less numerous than those seen on comparable sections treated with antisera directed against the m-GnRH (1076 and CRR11B73). In addition, many l-GnRH immunoreactive fibers were seen within the median eminence. No immunoreactivity was present in sections treated with lamprey-directed antisera (1467 and 1459) that were incubated with synthetic l-GnRH (10^{-6} M:24 hours at 4 ^{0}C), whereas, pre-incubation of lamprey antisera (1459) with a similar antigen excess (10^{-6}M) of m-GnRH (Sigma) had no effect on the immunocytochemical reaction, and yielded results that were comparable to the non-absorbed lamprey antisera. No immunoreactivity was present in sections treated with mammalian directed antiserum (1076) that was incubated with m-LHRH (10^{-6}M:24 hours at 4 ^{0}C). Our findings indicate that lamprey like GnRH can be found in human hypothalamus and median eminence, suggesting a possible role in the regulation of pituitary function. [1K11AG00295; HD19803; Great Lake Fish. Comm., NSF: DCB-8602907, Brain Bk MH/NS 31862.]

437.9 NEURONAL PERIKARYA IMMUNOREACTIVE WITH LAMPREY LHRH ANTISERUM PROJECT TO THE NEUROHYPOPHYSIS, THIRD VENTRICLE AND SURFACE OF THE BRAIN OF PETROMYZON MARINUS. S. A. Sower, E. L.P. Anthony and J. C. King. Dept. of Zoology, University of New Hampshire, Durham, NH, 03824, Dept. of Biology, Rhode Island College, Providence, RI, 02908, Dept.of Anatomy and Cellular Biology, Tufts University School of Medicine, Boston MA, 02111.

The role of LHRH in mammalian reproduction has been studied extensively; however, the role of a structurally different, but related decapeptide, is not well characterized in the most primitive class of vertebrates, Agnatha. Utilizing an antiserum directed to the recently characterized lamprey LHRH, we examined immunopositive neuronal perikarya and fibers in sections from the brain of the sea lamprey, Petromyzon marinus, using the unlabelled peroxidase antiperoxidase method. Neuronal perikarya and fibers, immunopositive with an antiserum generated to lamprey LHRH, were also immunopositive with certain antisera generated to mammalian LHRH. Immunopositive neuronal perikarya were detected in an arc-shaped population extending from ventral to dorsal preoptic areas. Fibers from these cells projected to the neurohypophysis via the preoptico-hypophyseal tract, but in addition projected into the third ventricle. Additionally, some fibers coursed along the external surface of the brain and may also release LHRH into meningeal compartments. Since the molecular forms present within perikarya were immunoreactive with an antiserum to mammalian LHRH that requires the N-terminal pGlu and C-terminal amidated Gly, the presence of fully processed, mature decapeptide is indicated. No reaction product was detected in lamprey sections incubated with an antiserum to mammalian LHRH that requires the interior 4-8 amino acid sequence. This finding supports the structure reported for lamprey LHRH in which differences within the decapeptide occur in amino acids in positions 3, 5, 6, 7, and 8 [Sherwood et al, J. Biol. Chem. 261:4812, 1986]. Little or no immunoreactivity was detected with two antisera generated to the C- terminal extension peptide of human precursor LHRH. Thus the precusor for lamprey LHRH may be a different product of the mammalian gene or the result of expression of a different gene. This work was supported by NSF grants DCB-8602907 [SAS], DCB-8510370 [ELPA] and NIH grant HD19803 [JCK].

437.10 EFFECT OF SHORT PHOTOPERIOD DURING DEVELOPMENT ON THE GnRH NEURONAL SYSTEM IN THE DJUNGARIAN HAMSTER. S.M. Yellon, K.K. Hirata and S.W. Newman, Div. Perinatal Biology, Dept. Physiol., Loma Linda University, School of Medicine, Loma Linda, CA 92350, and Dept. Anatomy and Cell Biology, The University of Michigan, Ann Arbor, MI 48109.

As a consequence of exposure to short photoperiod reproductive development is markedly delayed in the male Djungarian hamster. Changes in the distribution or morphology of GnRH neurons are hypothesized to account for the inhibition of gonadotropin secretion and forestalled gonadal maturation associated with short days. In the present study, the distribution of GnRH neurons in the brains of postpubertal males was compared to that in hamsters with short photoperiod-induced delayed puberty. At 40 days of age, hamsters reared either in long days (16L:8D; n=4) or short days (10L:14D; n=4) were anesthetized and perfused with Zamboni's fixative. Brains were vibratome-sectioned (60 μm) from the caudal olfactory tubercle to the rostral mammillary bodies. Immunocytochemistry for GnRH was performed on every section with specific antiserum (LR-1; gift of R. Benoit) and a Vectastain ABC-GO kit. Alternate sections were counterstained with cresyl violet and neuroanatomical locations of bipolar and unipolar types of GnRH soma assessed. In long days, most GnRH-immunoreactive perikarya were found in the medial preoptic area, diagonal band of Broca and lateral hypothalamus. Fewer cells were located in the lateral preoptic area, lateral septum, and lateral borders of the anterior hypothalamus. Typical fusiform bipolar GnRH neurons outnumbered ovoid unipolar perikarya 3:2 and 2:1 in some regions. In brains from short-day reared hamsters, most GnRH neurons were found in the same neuroanatomical locations as in males in long days. However, a 62% reduction in the total number of immunoreactive perikarya (136 ± 14 vs 356 ± 37, short vs long days) was accompanied by a shift in the proportion of bipolar to unipolar GnRH soma (about 1:1). The decrease in GnRH cell number was greatest in the diagonal band of Broca and lateral hypothalamus (>75%) with about a 50% fall in other brain regions. In nearly all brain regions, the number of a subpopulation of GnRH cells, bipolar and unipolar neurons with relatively thick processes, remained the same irrespective of photoperiod. period. Therefore, the percentage of GnRH perikarya with relatively thick processes doubled in hamsters under short compared to long days (57% vs 28%). These findings suggest that photoperiod-induced delayed puberty is associated with suppressed development of the GnRH neuronal system, and specifically, with decreased GnRH immunoreactive perikarya with thin processes. Areas such as the diagonal band of Broca and lateral hypothalamus may, in particular, be implicated in the photoperiod control of sexual maturation. (Supported by LLU Sch. of Med. and NIH NS #20629)

437.11 IMMUNOCYTOCHEMICAL DISTRIBUTION OF VASOACTIVE PEPTIDES AND TRANSMITTERS IN BOVINE EPICARDIAL CORONARY ARTERIES. G. Nilaver, F. Aguilar and B. Greenberg. Dept. Neurology and Cardiology, Oregon Health Sci. Univ., and Dept. Neurology, Good Samaritan Hospital and Medical Center, Portland, OR 97201.

Cardiovascular abnormalities are frequently seen in acute CNS catastrophies such as subarachnoid hemorrhage, and are believed to be caused by hypothalamic stimulation leading to an acute increase in sympathetic tone. The peptidergic complexity of the hypothalamus and the discovery of several perivascular peptide containing fibers has suggested a role for perivascular peptidergic neural networks in mediating some cardiovascular manifestations of intracranial disease. We have evaluated the perivascular distribution of known vasoactive peptides in bovine coronary arteries in order to better understand the neural regulation of coronary blood flow. Formalin fixed cryostat cut (50 um) cross sections of coronary arteries were immunocytochemically reacted with antisera to substance P (SP), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), neurotensin (NT), oxytocin (OT), vasopressin (VP) and atrial natriuretic factor (ANF) employing biotinylated protein A with avidin-biotin peroxidase in the pre-embedding staining technique. A few sections were also immunoreacted with antisera to the catecholamine synthetic enzymes, tyrosine hydroxylase (TH), dopamine B-hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT), and antisera to serotonin (5-HT). Immunoreactivity was confined to the adventitia and media of the blood vessels. Most of the fibers were observed to run in the adventitia-media junction. A moderate to dense plexus of NPY-, VIP-, and TH-reactive fibers ensheathed the vasa vasorum as they entered the coronary artery. The staining results are summarized below:

	adventitia	advent-media	outer media	mid media	inner media
SP	+	++	+++	+	-
VIP	++	+++	++	-	-
NPY	+++	+++	+++	++	-
NT	-	-	-	-	-
OT	-	-	-	-	-
VP	-	-	-	-	-
ANF	-	-	-	-	-
TH	+++	+++	+++	+	-
DBH	++	++	++	-	-
PNMT	-	-	-	-	-
5-HT.	-	-	-	-	-

These observations support the concept that hypothalamic regulation of coronary circulation by SP, VIP, NPY and noradrenaline is mediated via perivascular nerves. Other agents may gain access to receptors in coronary vessels upon release from the hypothalamus (NT, OT, VP, ANF) or atrium (ANF). The role of the peptidergic fibers in the vasa vasorum remains to be determined.

437.12 C-TERMINAL FRAGMENT OF PROPRESSOPHYSIN IN HYPOTHALAMIC CELLS OF THE HOMOZYGOUS BRATTLEBORO RAT: IS THE INCREASE IN THEIR NUMBER DURING LIFE DUE TO GENE CONVERSION? F.W. van Leeuwen, E.M. v.d. Beek, A.R. Caffé, M.A. Seger* and J.P.H. Burbach* (SPON: ENA). Netherlands Institute for Brain Research, Amsterdam; *Rudolf Magnus Institute for Pharmacology, Utrecht, The Netherlands.

In a number of studies homozygous Brattleboro rats (HO-DI) appeared to contain a very low number of cells in the supraoptic and paraventricular nucleus able to synthetize and transport propressophysin. These intensely stained cells were detected with various antisera directed against the C-terminal fragment of propressophysin (CPP) and vasopressin (Richards et al., Neuroscience 16, 617, 1985; Mezey et al., Neuropeptides 7, 79, 1986; Van Leeuwen et al., Brain Res. 379, 171, 1986). Comparison of our cell counts with those of Richards et al. (1985) revealed a difference (52.2±3.3 versus 15.4±1.5). This difference was explained by variations in the immunocytochemical procedures (e.g., vibratome-paraffin sections), whereas Richards et al. used female rats and we males. Using an antiserum raised against synthetic rat CPP fragment (22-39), we found even more cells in a pilot study (n=96). The increase in the number of cells might be caused by this particular antiserum. We therefore again examined a group of young HO-DI rats (age: 3 months), and a surprisingly low number of cells was found (19.2±0.8) compared with that reported by Richards et al. The antiserum as a cause for increased cell numbers could thus be excluded. When we then reexamined the age of the rats used in our first study, it appeared that we had used rats in the age of 8 months and older. It is therefore very likely that the age of the rat is of importance to the number of cells.

A theoretical explanation of the presence of CPP immunoreactivity in the HO-DI rat was recently given by Ivell (Neuropeptides and their peptidases, Turner, A.J. ed., Ellis-Horwood, 1987). Ivell proposed that in the HO-DI strain gene conversion might take place in these solitary cells. Two related neighbouring but non-allelic genes might transfer information in a non-reciprocal fashion, thereby becoming more similar (Kourilsky, Trends in Genet. 3, 60, 1986). For the HO-DI rats gene conversion in a minority of cells means that they become heterozygous and able to synthetize a more complete propressophysin molecule (in addition to the mutant precursor). Ivell also discussed the time of gene conversion and suggested that this event could occur within a somatic chromosome. The increase in the number of CPP-immunoreactive cells during life supports this idea.

437.13 NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY IN THE HYPOTHALAMUS OF THE YOUNG RHESUS MONKEY (MACACA MULATTA). J. Tigges, J. K. McDonald, and M. Tigges. Yerkes Regional Primate Research Center, and Depts. of Anatomy and Cell Biology, and Ophthalmology, Emory University, Atlanta, GA 30322.

The distribution of NPY-like immunoreaction product within the hypothalamus was investigated in 6 monkeys ranging in age from 1 to 13 months. The monkeys, which were not treated with colchicine, were deeply anesthetized and perfused with saline, followed with a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer, and with 5% sucrose in the same buffer. The brains were cut in a cryostat in the transverse plane at 40 um. Every fifth section was incubated in rabbit antiserum to porcine NPY (NPY-3 McDonald) and processed for PAP histochemistry. Adjacent sections were stained with cresyl violet acetate to identify cytoarchitectonic boundaries as described in the atlas of the rhesus monkey hypothalamus by R. Bleier (The University of Wisconsin Press, 1984).

Immunoreactive somata occurred only in two locations; the bed nucleus of the stria terminalis contained few stained somata, whereas the arcuate nucleus displayed numerous labeled cells. In contrast to a previous finding in the squirrel monkey (Smith et al., JCN 236:71, 1985), no labeled somata were observed in the supraoptic and paraventricular nuclei of the rhesus monkey. Immunoreactive varicose axons were distributed throughout the hypothalamus, but their concentration varied markedly. The smallest number of labeled axons was located in the anterior portion of the supraoptic nucleus and the various subnuclei of the mammillary body. A moderate number of fibers were seen in the lamina terminalis, anterior, lateral, posterior, and dorsal hypothalamic areas, in the subventricular, suprachiasmatic, and ventromedial nuclei, and in the infundibular stalk. The largest concentration of varicose immunoreactive axons and punctate label occurred in the bed nucleus of the stria terminalis, in the medial preoptic area, and in the periventricular, paraventricular, arcuate, and perifornical nuclei. In many instances, particularly in the lateral hypothalamus, punctate label appeared along non-reactive dendrites and somata, suggesting synaptic contact between presynaptic NPY-positive axons and unlabeled processes and cell bodies. Numerous immunoreactive fibers penetrated the ependymal lining of the third ventricle suggesting that these fibers might release NPY directly in the CSF to affect distant targets.

In view of these anatomical results and previous reports which indicate central effects of NPY on the secretion of luteinizing hormone, growth hormone and insulin in rats (McDonald et al., PNAS 82:561, 1985; Moltz and McDonald, Peptides 6:1155, 1985), we speculate that NPY may play a significant role in the hypothalamic regulation of hormone secretion in primates. (NIH grants RR-00165, EY-06001, HD-19731, HD-00727, and The March of Dimes 5-524.)

437.14 OXYTOCINERGIC CELLS IN THE MEDIAL PREOPTIC AREA AND PERIFORNICAL REGION: AN IN SITU HYBRIDIZATION AND IMMUNOHISTOCHEMICAL STUDY. P.J. Brooks[1], P.K. Lund[2], J.D. Caldwell[3] and C.A. Pedersen[3],(SPON: M. Numan). The Curriculum in Neurobiology[1], and the Departments of Physiology[2] and Psychiatry[3], University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

Oxytocin immunostaining of cells in the medial preoptic area (MPOA) has been described previously by ourselves as well as others. In the present study, in situ hybridization histochemistry was used to investigate synthesis of oxytocin mRNA by cells in the MPOA and other regions of the rat brain.

Five micron frozen sections were prepared from 4% paraformaldehyde-0.2% glutaraldehyde perfused female rat brains. In situ hybridization was performed essentially as described by Han et al. (J. Neurosci. Res. 16: 97). The probe was a P^{32}-labelled synthetic oligodeoxyribonucleotide (38mer) complementary to the last 36 bases of exon C and the first two bases of the 3' untranslated region of the oxytocin mRNA.

Within the classical magnocellular nuclei, hybridizing cells were detected in the dorsal region of the supraoptic nucleus, as well as in the anterior commissural nucleus. In the paraventricular nucleus, hybridizing cells formed a shell pattern, characteristic of oxytocin producing cells in this nucleus, around a core of unlabelled and presumeably vasopressinergic cells.

Within the MPOA, labelled cells were most prevalent dorsally, under the crossed anterior commissure. However, scattered labelled cells were also seen in the ventral and periventricular regions of the MPOA. Labelled cells were also detected in the perifornical region, ventral and lateral to the fornix at the level of the MPOA. The hybridization signal was greatly decreased by RNAse pretreatment, and completely abolished by prehybridization with excess cold probe. In all brain regions where hyridizing cells were detected, oxytocin immunostaining cells were also detected in vibratome sections. We are currently using a combined immunohistochemical in situ hybridization procedure on vibratome sections to confirm the presence of both oxytocin peptide and mRNA in the same cells in the MPOA, perifornical region, and other extrahypothalamic sites.

This work was supported by NIH grants NS09914, 14016150, NS 23804 and NIMH grant MH 33127.

437.15 CO-EXISTENCE OF MET^5-ENKEPHALIN-ARG^6-GLY^7-LEU^8 AND OXYTOCIN IMMUNOREACTIVITY IN THE MAGNOCELLULAR NEURONS OF THE RAT HYPOTHALAMUS. Y.N. Wang, J.K. Chang, and D. Chang. Peninsula Laboratories, Inc. Belmont, CA 94002.

Immunohistochemical studies have reported that opioid met^5-enkephalin (met-enk) appears to be co-localized with oxytocin in the hypothalamic magnocellular neurons as well as in the nerve terminals of the neurohypophysis. In addition, functional studies have indicated that met-enk inhibits the release of oxytocin from the nerve terminals of the neurohypophysis. Our previous studies have demonstrated that opioid octapeptide met^5-enkephalin-arg^6-gly^7-leu^8 (met-enk-arg-gly-leu), derived from the precursor molecule proenkephalin A, was widely dispersed in the magnocellular neurons, with distribution parallel to that of vasopressin in the hypothalamus of the rat. (Wang & Wyatt, *Society for Histochem. and Cytochem. Abstract,1,* 1986). The aim of the present study, by use of immunohistochemistry, is to investigate the co-existence of met-enk-arg-gly-leu with pituitary hormones, oxytocin and vasopressin, in the hypothalamic neurons. The rats were injected with colchicine (50 µgm in 25 µl of saline) into the lateral cerebral ventricle before they were perfused. The brains were cut in a cryostat at 5-10 µm. The tissue sections were processed by the indirect immunofluorescence technique. The primary antisera were used at a dilution of 1:200 to 1:500. The secondary antiserum was goat anti-rabbit-FITC at a dilution of 1:50. Specificity was assessed by incubating tissue sections with the antiserum preabsorbed with 10 µM synthetic met-enk-arg-gly-leu. The majority of the met-enk-arg-gly-leu-like immunoreactive nerve cell bodies were located in the paraventricular nucleus and the supraoptic nucleus while the scattered neurons were present in the periventricular nucleus, the arcuate nucleus and the ventromedial nucleus. Immunoreactive nerve fibers were generally found in the same regions as the cell bodies and additional dense collections were seen in the median eminence and posterior pituitary. Staining of the serial sections (5 µm) demonstrated that met-enk-arg-gly-leu co-existed with oxytocin in the magnocellular neurons in the paraventricular and supraoptic nuclei, but not with vasopressin, even though their distributions were similar. The extensive presence of met-enk-arg-gly-leu in the hypothalamus and the posterior pituitary and the co-existence of this octapeptide with oxytocin in the hypothalamic neurons suggest that enkephalins may play an important role in the regulation of oxytocin secretion in the hypothalamo-hypophysial system.

437.16 LOCATION OF MORPHINE-MODULATING NEUROPEPTIDES IN THE RAT HYPOTHALAMUS AND NEUROHYPOPHYSIS. L.Kivipelto, P.Panula, E.A.Majane and H.-Y.T.Yang. Dept. of Anatomy, University of Helsinki, 00170 Helsinki, Finland and Lab. Preclin. Pharm., NIMH, St.Elizabeth's Hosp., Wash., D.C. 20032.

Two peptides that cross-react with an antiserum against the molluscan tetrapeptide Phe-Met-Arg-Phe-NH_2 (FMRF-NH_2) were recently isolated from bovine brain extract (Yang et al., Proc.Natl.Acad. Sci.USA 82:7757-7761, 1985). These peptides have an attenuating effect on morphine-induced analgesia when injected intracerebroventricularly in rats. Antisera against the two peptides, an octapeptide Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 (F-8-F-NH_2) and an octadecapeptide Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-NH_2 (A-18-F-NH_2) were raised in rabbits and characterized with standard radioimmunoassay, immunohistochemical blocking controls and model experiments with synthetic peptides on nitrocellulose filter.

Adult Sprague-Dawley rats were perfused under sodium pentobarbital anesthesia through the left ventricle with 4% paraformaldehyde. Some rats were given colchicine i.c.v. 24 hours prior to perfusion. 60um thick cryostat section were cut, incubated with the specific antisera and the immunoreactivity was visualized by either the FITC or the PAP method.

One immunoreactive neuronal cell group was detected in the hypothalamus of colchicine treated rats. It was located in the periventricular hypothalamic area, extendig from the dorsomedial nucleus to the periventricular and paraventricular nuclei. Nerve fibres were seen in the median eminence and the arcuate nucleus. No immunoreactivity was seen in the anterior and intermediate lobes of the pituitary, whereas in the posterior lobe immunoreactive nerve fibres and terminals were detected throughout the area. In all the areas above the immunoreactivity was completely abolished when the antisera were pre-incubated with the corresponding synthetic peptides F-8-F-NH_2 and A-18-F-NH_2. Pre-incubation of the antisera with neuropeptide Y (NPY) did not affect the staining.

The results suggest that there are neurons in the hypothalamus and neuronal fibres in the median eminence and neurohypophysis that contain F-8-F-NH_2 and A-18-F-NH_2-like peptides. This immunoreactivity cannot represent FMRF-NH_2 since this peptide does not appear in mammalian tissues. The pre-absorption controls showed that the antisera did not react with NPY. The neuronal cell bodies in the hypothalamus may project down to the neurohypophysis and possibly modulate its function. However, there may also be projections originating from these cells more caudally to the medulla oblongata, which remain to be studied with tract tracing methods.

437.17 **LOCALIZATION OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH) IN THE PORCINE HYPOTHALAMUS BY IMMUNOCYTOCHEMISTRY.** **R. D. Kineman*, L. S. Leshin, J. W. Crim*, G. B. Rampacek* and R. R. Kraeling*. University of Georgia, Athens, GA 30602 and USDA-ARS, Athens, GA 30613.**

Three mature female pigs were anesthetized with halothane and, then via the common carotid arteries, perfused sequentially with sodium citrate and Zamboni's fixative. Immediately after starting perfusion, the head was severed from the body to allow isolated perfusion. After perfusion, the brain was removed and the hypothalamus and the septal-preoptic area were dissected and placed in 20% sucrose: Zamboni's fixative for 24 h at 4°C then frozen and stored at -80°C until analysis. Sections (56 and 70 µm) were cut transversely with a cryostat and were collected in phosphate buffer containing bacitracin. LHRH was localized by using indirect biotin-avidin horseradish peroxidase immunocytochemistry. Free floating sections were treated sequentially with sodium periodate and sodium borohydride to eliminate reactive aldehydes of the fixative, normal goat serum to decrease nonspecific binding, rabbit anti-LHRH, biotinylated goat anti-rabbit IgG, horseradish peroxidase-avidin D, and diaminobenzidine in the presence of hydrogen peroxide. A dark brown precipitate formed at the site of the antigen-antibody reaction. Controls for demonstrating specificity of the reaction included adjacent sections treated with primary antiserum preabsorbed with LHRH or by substitution of normal rabbit serum for anti-LHRH. Control sections lacked positive brown stain characteristic of experimental sections.

Two antibodies (Dr. Ramirez CR11B73 and Miles-Yeda 00100) yielded positive staining. Bipolar and multipolar LHRH perikarya were identified throughout the medial ventral septal-preoptic area, but were more densely packed in the area of the suprachiasmatic nucleus and the medial basal hypothalamus: predominantly the arcuate nucleus. Some perikarya were closely associated with the third ventricle in the area of the periventricular nucleus. Fibers containing LHRH were found from the medial ventral septal-preoptic area to the mammillary region. Densely packed fibers were located in the ventral medial basal hypothalamus and median eminence. LHRH fibers along the third ventricular wall appeared to extend dorsal-ventrally toward the median eminence. These initial findings suggest that the distribution of LHRH staining within the porcine hypothalamus differs in detail from those reported for other large domestic species (ovine, bovine and equine).

437.18 COMPARISON OF ANGIOTENSIN II LEVELS IN MICRODISSECTED CNS NUCLEI OF THE DEVELOPING DAHL HYPERTENSIVE RAT AND ITS NORMOTENSIVE CONTROL. L. Lark* and J. A. Weyhenmeyer. Neural and Behavioral Biology Program and College of Medicine, University of Illinois, Urbana, IL 61801.

Neurogenic mechanisms have been implicated in the pathogenesis of hypertension. The Dahl salt sensitive (DS) rat was inbred on the basis of its tendency to develop hypertension when on a high salt diet and the Dahl salt resistant (DR) rat on the basis of its resistance to such treatment. A hypersensitive brain angiotensin II (ANG II) system has been suggested by the demonstration of a greater pressor response to intraventricular administration of ANG II in the DS versus the DR rat (Ikeda et al., Clin. Sci., 55:225S, 1978). The purpose of the present study was to measure levels of ANG II in microdissected brain nuclei of the developing DS and DR rat.

Although the DS and DR rats were maintained on a diet with normal NaCl levels, DS rat pressures (150 ± 16.6 mm Hg) were significantly higher ($p < 0.001$) than DR rat pressures (119 ± 9.7 mm Hg) at 10 weeks of age. Therefore, pressures were elevated but not to the debilitating and often lethal extent observed when DS rats are fed an 8% salt diet.

Brain nuclei from 4 and 10 week male DS and DR rats were prepared as previously described (Lark and Weyhenmeyer, Neurosci. Abstr., 12:1041, 1986). Briefly, nuclei were micropunch dissected from 300 µm brain slices. Prepared samples were analyzed by RIA using an ANG II antibody. Animals were fed a .56% NaCl diet and blood pressures were measured using the indirect tail cuff method.

Preliminary results indicate that fmole levels of ANG II (per mg wet tissue weight) are present in brain nuclei of all rats studied. At 4 weeks of age, highest levels of ANG II in both the DS and DR rats were found in circumventricular nuclei (OVLT, SFO, and AP), the anterior and paraventricular (PVN) nuclei of the hypothalamus, and the A2 area of the brainstem. DR rats also displayed high levels in the posterior hypothalamus (PH). In most of these areas, 4 wk DR rat tend to have slightly higher AII levels than the 4 wk DS rat. A similar distribution of AII was found in the 10 wk DS and DR animals. AII levels were found to decrease with age in several of the DR nuclei including the OVLT, SFO, AP, PVN, PH, locus coeruleus, and parabrachial nucleus. AII levels in DS nuclei tended to remain the same with age.

These results suggest that the brain AII system may function differently in the DS and DR strains. Such differences could relate to the DS rat's development of hypertension or the DR rat's resistance to high salt treatment.

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437.19 LUTEINIZING HORMONE-RELEASING HORMONE IN THE BRAIN AND NERVUS TERMINALIS OF THE GRAY SHORT-TAILED OPOSSUM (MONODELPHIS DOMESTICA). Marlene Schwanzel-Fukuda, Barbara H. Fadem[1], Maria Soledad Garcia* and Donald W. Pfaff. The Rockefeller University, New York, New York and [1]The New Jersey School of Medicine and Dentistry, Newark, New Jersey.

The brains of 8 adult, and whole heads of 18 neonatal (days 0-12) gray opossums were fixed in Bouin's solution, embedded in Paraplast and serial 6µm sections cut and mounted onto glass slides. The immunocytochemical localization of luteinizing hormone-releasing hormone (LHRH) and luteinizing hormone (LH) was carried out with unlabeled antibody enzyme procedures. Localization with antiserum to LHRH (LR1, Dr. Robert Benoit; #633, Dr. Lothar Jennes) revealed a distribution of LHRH in cells and fibers in the brain and in ganglia of the nervus terminalis of the adult and neonatal gray opossum similar to that seen in several species of placental mammals. LHRH-containing cells and fibers were seen in the medial septal nuclei, nucleus and tract of the diagonal band and olfactory tubercle. An unusual observation was the apparent absence of LHRH cells in the hypothalamus in both the adult and neonate. LHRH-reactive fibers were seen in these regions and were numerous in the median eminence. The nervus terminalis in the opossum showed several distinctive characteristics. Immunoreactive and non-reactive cells, observed in ganglia along the peripheral and intracranial course of this nerve, were accompanied by thick fascicles of LHRH fibers and frequently seen adjacent to blood vessels. The LHRH-reactive fibers in the central roots of this nerve formed a compact bundle which coursed obliquely from clusters of ganglion cells on the ventromedial surface of the olfactory bulbs into the septum. From the triangular nucleus of the septum, these LHRH fibers ran down and around the rostral face of the anterior commissure and fanned out into the medial preoptic area. As previously observed in the fetal rat and guinea pig, LHRH was detected in ganglion cells of the nervus terminalis of the newborn gray opossum, preceding its detection in any other area of the brain and the appearance of immunoreactive LH in the anterior pituitary. The paucity of LHRH neurons visualized in the in the hypothalamus may be due to rapid synthesis and release of the decapeptide or to differences in the makeup or conformation of the LHRH molecule in hypothalamic neurons in contrast to that seen in the nervus terminalis and other regions of the brain and inability of the antisera used in this study to recognize a particular configuration of the peptide. The presence of LHRH in the nervus terminalis of a marsupial, similar in distribution and time of appearance in development to that seen in placental mammals indicates that the LHRH system of the nervus terminalis is a phylogenetically ancient system. Supported by NIH NS19662-04 and funds from the Whitehall Foundation (MS-F).

438.1 **EFFECTS OF NEUROTOXIC LESIONS IN THE LATERAL SEPTAL AND HIPPOCAMPAL AREAS ON THE IMMUNE RESPONSE OF FEMALE RATS.**
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Kainic acid (KA) lesions in the lateral septal area of female rats produce a variety of psychoneuroendocrine changes which include an increase in ovarian compensatory hypertrophy, a decrease in female sexual behavior, an increase in body weight and an attenuation in the anorexic effects of estrogen on food intake and body weight (P.B.&B. 24, 107, 1986). One common aspect of these lesion effects is a generalized reduction in the physiological and behavioral effects of estrogen. Estrogen has been shown to modulate the immune system and typically reduces cellular immunity and facilitates humoral immunity (Immunopharmacol. 11, 47,1986). Thus, if neuroendocrine regulatory processes are integrated with the neural control of the immune system, lesions in the lateral septum may also produce changes in the immune response of female rats. In two experiments, we have tested the effects of electrolytic and KA lesions in the lateral sepatal area of female rats and have compared the effects of KA lesions in the lateral septal area with KA lesions in the hippocampal region on antibody production following immunization with 100 µg ovalbumin in complete Freund's adjuvant. Blood samples were collected on days 0, 7, 14, 17 and 28 following immunization in the first experiment and on days 0, 7, 14, and 28 in the second study. All animals were boosted with ovalbumin on day 14. The IgG, IgA and IgM antibody titers were then measured by an enzyme-amplified ELISA assay. KA lesions in the lateral septal area produced extensive cell loss in this region and produced a concurrent loss of cell bodies in the CA 3 - CA 4 hippocampal areas. Compared to sham and electrolytic septal lesioned rats, KA lesions of the lateral septal area significantly reduced IgG titers on days 7 and 17 with a trend for day 14. Similarly, the IgA and IgM titers were significantly lower on days 7, 14, and 17 for the rats with KA lesions in the lateral septal area. In addition, the KA septal lesioned rats gained more body weight and had higher thymus weights, relative to the electrolytic lesioned and sham groups. Adrenal, ovarian and spleen weights were comparable for all groups. In the second study, the decrease in immune response of rats with KA lesions in the lateral septal area was replicated and relative to controls, IgG, IgA and IgM titers were significantly lower on days 7 and 14. In contrast, the rats with KA lesions in the hippocampal area showed significantly elevated IgG titers on day 14, lower IgA titers on day 28 and higher IgM titers on day 7, relative to the sham group. When the rats were reimmunized with a new antigen, beta-lactoglobulin, the inhibitory and facilitory effects of septal and hippocampal lesions on antibody production, respectively, were still present, indicating that these effects are sustained. Thus, the decrease in immune response of rats with KA lesions in the lateral septal area cannot be accounted for by the concurrent hippocampal damage typically associated with these neurotoxic lesions in that the effects of hippocampal lesions are opposite to those of KA lesions in the septal area. These results suggest that neuroendocrine and neuroimmune regulatory processes are modulated by a common control system. Supported by MRC of Canada.

438.2 IMPAIRED ANTIBODY PRODUCTION ASSOCIATED WITH STRESS: CIRCADIAN INFLUENCES.
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The immune response of the intact organism is significantly affected by circadian rhythms with regard to both in vitro measures and in vivo responses. How circadian rhythms might influence the impact of uncontrollable aversive events on the immune response is not well defined. The effects of exposure to uncontrollable tail shock on the production of antibodies to a specific antigen, keyhole limpet hemacyanin (KLH), was studied in pathogen free adult male Sprague Dawley rats. Twenty-four animals were maintained on a reversed day/night cycle (LD 12:12) and tested during their active phase (0900 - 1200 hrs). A second group (N = 24) was maintained on a normal day/night cycle (LD 12:12) and tested during their inactive phase (0900 - 1200 hrs). All animals in each group were immunized twice with a subcutaneous injection of KLH (1.25 mg) at the base of the tail at one week intervals. Shock exposure immediately followed each immunization. Sixty days following the first injection in the absence of further shocks, a secondary response was elicited with a third injection of KLH (1.25 mg). Blood samples were obtained from the tip of the tail at the time of each immunization and at one week intervals for two weeks following the first and third immunizations. Twelve subjects from the active and inactive phases were lightly restrained and exposed to 100 5 sec inescapable tail shocks increasing in intensity (from .8, 1.0, 1.3, to 1.6 ma)every 25 shocks. The remaining twelve subjects in each group served as home cage controls and were immunized but not shocked. Serum levels of IgG antibodies to KLH were determined by enzyme linked immunosorbent assay (ELISA). Animals exposed to shock and immunized with KLH during the active phase of their cycle showed a reduced primary and secondary response to KLH relative to control animals immunized at the same time. In contrast, animals shocked during the inactive phase of their cycle did not differ from controls immunized at the same time. These observations are suggestive of an important influence of some aspect of the circadian rhythm on the impact of uncontrollable aversive events on the immune response. Supported in part by Office of Naval Research Contract Number N0014-85-K-0411 and PHS grant MH37373.

438.3 **CO-INDUCTION OF IMMUNOSUPPRESSION AND GASTRIC EROSION BY STRESS IN RATS.**
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Among the pathophysiological conditions observed following exposure to stressful stimuli, it has been reported that stress alters the normal functioning of the immune and neuroendocrine systems and produces gastrointestinal (GI) erosions. The studies reported here are the initial experiments in a series of investigations aimed at assessing a possible link between stress-induced GI erosions and alterations in immune function. Three chronic stress regimens were compared for effects on the integrity of the GI mucosa and the immune system. Since glucocorticoids have important immunoregulatory actions and play a role in stress-induced gastric erosions, changes in plasma corticosterone levels were also measured.

Male Sprague-Dawley rats were exposed to one of the following: immobilization stress (IMM; 3 hr/day for 10 days), performance stress (PS; avoidance or escape of electric footshock dependent upon performance of a task, 24 hr/day for 14 days, Anderson et al., Pharmacol. Biochem. Behav. 26: 829-833, 1987) and activity wheel stress (AWS; access to food limited to 1 hr/day, continuous access to activity wheel for 7 days, Manning et al., Physiol. Behav. 21: 269-274, 1978). IMM was characterized by a moderate reduction in spleen size (1.6 ± 0.1 vs 2.3 ± 0.1 mg/g body weight for control rats) and thymus size (1.4 ± 0.1 vs 2.0 ± 0.1 mg/g body weight for control). However, there were no changes in the proliferative responses to mitogenic stimuli (Concanavalin A, 0.25 to 2.0 µg/ml) of splenocytes from IMM rats. No significant changes in spleen or thymus size, or lymphocyte proliferation were found in PS rats. In contrast, AWS produced a marked reduction in mitogen stimulated lymphocyte proliferation (e.g. 6908 ± 4557 cpm vs 104752 ± 10911 cpm for control at 0.5 µg/ml Con A). Spleen (1.5 ± 0.3 vs 2.6 ± 0.1 mg/g body weight for control) and thymus (0.8 ± 0.2 vs 1.7 ± 0.2 mg.g body weight for control) weight were also reduced by AWS. The immunosuppressive effects of AWS were most prominent in animals demonstrating a decline in running activity, body weight and food intake. There was also a pronounced increase in the incidence of gastric erosions in AWS animals (21.9 ± 8.6 vs 0.1 ± 0.1 lesions/stomach in controls), while gastric morphology was unaffected by IMM or PS. There was no significant degree of duodenal erosion with any of the stress regimens. However, among those AWS animals in which small duodenal lesions were found, the greatest degree of immunosuppression was observed. Circulating corticosterone levels were increased with IMM (338%) and AWS (231%) relative to control, but were not changed with PS. These results indicate that of the three chronic stress regimens tested, only the AWS regimen affects changes in immune competence, certain neuroendocrine parameters and the integrity of the gastric mucosa. Furthermore, these effects were not directly correlated with altered corticosterone levels. Thus, the AWS model appears to be suitable for assessing a possible link between stress-induced immunosuppression and erosion of the GI lining. (Work supported in part by a National Research Council Research Associateship to H.B.).

438.4 THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN THE IMMUNE RESPONSE TO STRESS.
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There is evidence that the nervous system and stress can modulate immune function. One possible mediator of this neuroimmune communication is the autonomic nervous system. We therefore evaluated the effect of sympathetic ablation on stress-induced immune suppression in rats. Rats were stressed using a conditioned emotional response paradigm. After sacrifice peripheral blood was taken for measurement of mitogen induced lymphocyte proliferation (stimulation index, SI). Sympathectomy was accomplished by surgical adrenal medullectomy combined with weekly injections of 6-hydroxydopamine (50mg/kg, i.p.), a specific noradrenergic neurotoxin. Sympathectomy was documented by determination of plasma epinephrine levels at the time of sacrifice, which were <1% of control animals Control, stressed rats showed profound suppression of the SI compared to unstressed rats, 1.40 ± 0.25 vs 7.28 ± 1.24 (P<.01). Unstressed, sympathectomized rats also depressed the SI compared to control, unstressed rats 2.90 ± 0.76 vs 7.28 ± 1.24 (P<.01). Sympathectomy did not block the effect of stress, however, as further depression of the SI occurred in stressed, sympathectomized rats, 0.58 ± 0.26 vs 2.90 ± 0.76, (P<.01). Plasma corticosterone levels were not different in sympathectomized rats compared to controls, but rose significantly with stress.

We conclude that 1.) stress suppresses mitogen stimulation of lymphocytes in rats. 2.) sympathectomy also induces immune suppression. 3.) the effect of stress on the immune system is not mediated by the sympathetic nervous system.

438.5 **STRESSOR ASSOCIATED CUES INFLUENCE IMMUNE FUNCTION**, J.Irwin, S. Livnat*, and R.Ader, Queen's University, Kingston, Canada, K7L 3N6 and University of Rochester, Rochester, N.Y., 14642.

A variety of stressors have been shown to reduce the effectiveness with which the immune system defends the host from disease. For example, a number of laboratories have demonstrated that lymphocyte proliferation was inhibited following exposure to inescapable shock, cold, and other physical stressors. Similarly, we have previously reported that Natural Killer (NK) cell lytic activity was attenuated in mice exposed to inescapable footshock or tailshock. The results of the present series of experiments suggest that the extent to which these stressors influence NK activity may depend on the environmental context in which the stressor is administered.

In an initial experiment, male C3H/HeJ mice were individually placed in shock boxes and exposed to one of two inescapable footshock protocols during a 1 hour period. Control animals were placed in adjacent boxes, but shock was withheld. Groups were subdivided and animals were sacrificed either immediately or 24 hours later for determination of splenic NK activity. Exposure to footshock did not significantly reduce NK activity in C3H/HeJ mice. Surprisingly, however, in non-shocked mice, NK activity was markedly increased 24 hrs following the end of the session. These control animals had been handled and placed in the shock apparatus at the same time as other animals were being exposed to the actual shock. Consequently, the cues associated with the stressor (i.e. odours, sounds) may have served to provoke these changes in NK. Additional experiments were then undertaken to test this hypothesis.

In contrast to mice which were neither handled nor exposed to the shock environment (Home Cage Control), non-shocked mice exposed to the apparatus again exhibited an increase in NK activity 24 hrs after handling. In a separate experiment, Home cage controls were compared to mice exposed to footshock or to one of 2 non-shocked conditions. That is, one group was placed in the no shock apparatus at the same time as other animals were receiving shock (No Shock With), while the other group was segregated and exposed to the apparatus in a separate room (No Shock Alone). Consistent with the earlier results, only those animals exposed to the environment in which animals were being shocked (No Shock With) exhibited an increase in splenic NK activity.

These data suggest that the responses of animals to a stressful situation may serve as signals to other animals that a threat is present. It may be speculated that in anticipation of such a threat, physiological changes occur which would prepare the organism to respond to the environmental challenge. Consequently, the enhancement noted in NK activity may be the byproduct of increased sympathetic activation, or it may be an adaptive response in the face of possible physical insult. Although the nature of the cues remains to be determined, these data do indicate that the environmental context in which a stressor is applied may be an important determinant of the immunological consequences of stress exposure.

438.6 **RELATIONSHIP BETWEEN PSYCHOLOGICAL, NEURAL, ENDOCRINE AND IMMUNOLOGIC CHANGES INDUCED BY AVERSIVE STIMULATION.** D.T. Lysle*, B.S. Rabin*, A.R. Caggiula, R. Myers*, J. Cunnick*, S.M. Antelman*, L.H. Epstein* and H. Fowler* Depts. of Pathology, Psychology, Psychiatry and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, Pa. 15260

Our research program is designed to systematically investigate the relationship between stress and immunocompetence, starting with a parametric analysis of the effects of aversive stimulation on immune function. In our initial study, male Lewis rats received 4, 8 or 16 signaled footshocks (5 sec., 1.6 mA, V.I. 4 min.) in each of 1, 3 or 5 daily sessions. The mitogenic response to Con A was progressively reduced for both whole-blood and spleen lymphocytes with increasing numbers of shock presentations. However, the immunosuppression attenuated across sessions for the spleen lymphocytes, whereas the blood lymphocytes remained suppressed. The spleen and whole-blood lymphocytes also differed in the rate of recovery from shock experience. The spleen lymphocytes recovered within 24 hours, whereas the blood lymphocytes took between 48 and 96 hours to recover.

In a separate study designed to compare different environmental stressors and different physiological stress responses, 16 footshocks during a single 64-minute period produced a reduced responsiveness of splenic lymphocytes to PHA and Con A, an increase in peripheral corticosterone levels, and increases in DOPAC in the frontal cortex and nucleus accumbens, with no changes in dopamine levels. By contrast, rats subjected to an identical regimen of unsignaled, 120 db white noise did not differ from home-cage controls on immune, corticosterone or dopamine-DOPAC measures. The latter is consistent with companion studies in which 100 or 120 db of noise presented for 1 or 3 hours per day for 1, 3 or 12 days did not affect immune responsiveness of Lewis or Sprague Dawley rats to mitogen stimulation.

Our most recent investigations have found that a signal for shock produces a potent immunosuppressive effect. Furthermore, our preliminary data indicates that the effect follows the principles of classical conditioning. We have demonstrated both extinction of the immunosuppressive response to the signal for shock and a latent inhibition effect.

438.7 **A VIRUS ACTIVATED STRESS RESPONSE IN MICE: CEREBRAL BIOGENIC AMINES, PLASMA CORTICOSTERONE, AND LYMPHOCYTE PROLIFERATION.** A.J. Dunn, M.L. Powell*, J.M. Gaskin* and N.R. Hall. Department of Neuroscience, College Medicine, and Department of Infectious Disease, College Veterinary Medicine, University of Florida, Gainesville, FL 32610 and Department of Biochemistry, George Washington Univ. School of Medicine, Washington, DC 20037

We have investigated the potential use of a virus (Newcastle disease virus, NDV) as a stressor in mice. NDV, a chicken virus, is not infectious in mice, but invades cells and provokes an immune response. NDV elevates body temperature about 2°, maximal about 2 hours after NDV. In confirmation of previous reports, NDV (750 HA units IP) increased concentrations of plasma corticosterone, maximal at about 8 hours; but, in contrast to a previous report (Smith, Meyer, and Blalock, Science 218: 1311-12, 1982), this response was prevented by hypophysectomy. In our experiments, mice that failed to respond to restraint with an increase in plasma corticosterone, exhibited no response to NDV; whereas those mice that did show a corticosterone response to restraint or CRF, also exhibited an increase after NDV injection. Therefore, it is likely that the results of Smith et al. were due to the inclusion in their data of incompletely hypophysectomized mice.

NDV administration caused a somewhat variable activation of cerebral dopamine (DA), norepinephrine (NE) and serotonin (5-HT) metabolism, as determined by measurement of the amines and their catabolites. Concentrations of dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxy,4-hydroxyphenylethyleneglycol (MHPG), and 5-hydroxyindoleacetic acid (5-HIAA), and DOPAC:DA, HVA:DA, MHPG:NE, and 5-HIAA:5-HT ratios were increased in hypothalamus and brain stem of both intact and hypophysectomized mice. Amine catabolites were not altered in the prefrontal cortex. Free tryptophan was increased in all regions in all experiments. These neurochemical changes are consistent with the pattern observed following stressors, such as footshock or restraint, except that the prefrontal cortex DA metabolites were not changed.

NDV also produced a profound decrease (60-90%) in the proliferative responses of isolated spleen cells, in the presence of phytohemagglutinin (PHA), concanavalin A (Con-A), poke weed mitogen (PWM) and lipopolysaccharide (LPS). This apparent immunosuppression cannot be due to corticosterone because it also occurred in hypophysectomized mice in which plasma corticosterone concentrations did not change. These results indicate that administration of NDV to mice can initiate neurochemical and endocrine responses like those in stress, and can also cause immunosuppression. They are consistent with the hypothesis that a nonpathogenic antigen can be a stressor.

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438.8 STRESSOR EFFECTS ON THE PLAQUE FORMING CELL RESPONSE TO SHEEP RED BLOOD CELLS. S. Zalcman*, M. Richter* and H. Anisman. Psychology Dept., Carleton University, Ottawa, Ont. Canada, K1S 5B6 and Dept. of Microbiology and Immunology, Dept. of Pathology, Faculty of Health Science, University of Ottawa, Ottawa, Ont. Canada, K1H 8M5.

A series of experiments assessed the effects of stressors on the plaque forming cell (PFC) response in mice. At various intervals (0, 24, 72 or 95 hours) following administration of a submaximal dose of antigen (sheep red blood cells; 10^6 cells/ml, ip), CD-1 mice were exposed to 360 shocks of 2 sec duration (150 uA). Spleens were taken 96 hr following antigen administration and plaque formation determined. A marked reduction (70%) of plaque formation was noted in mice that had been stressed 72 hr after antigen administration, while no effect was evident in mice that were exposed to the stressor 0, 24 or 95 hr after antigen treatment. This effect was not related to the time between stressor exposure and spleens being taken, since a reduction in plaque formation was also evident when the number of PFC's were determined120 hr after antigen administration. Thus, the time between application of the antigen and exposure to stressor was fundamental in determining the immunological response. Additionally, the magnitude of the PFC suppression was directly related to the number of shocks mice received. Neither10 nor 30 shocks affected plaque formation, while a marked reduction (30%) was evident following 60 shocks. Contrary to the PFC reduction in virtually all animals following 360 shocks, the effects of the 60 shock treatment appeared to be of an all-or-none nature, in that profound suppression of plaque formation was only evident in about half the mice.

In a subsequent experiment, uncontrollable and controllable shock were found to be equally effective in suppressing plaque formation. In contrast, the same stressor parameters were previously shown to influence central norepinephrine concentrations. Thus, the occurrence of the suppression of PFC's was not related to the organism's ability to contend with the stressor through behavioral means. It is provisionally suggested that stressful events may profoundly influence the immune response, but that the time between antigen administration and subsequent exposure to a stressor is critical in determining whether such an effect will be evident.

438.9 AGGRESSIVE BEHAVIOR AND DOMINANCE MODIFY IMMUNE RESPONSES IN MICE. C-A. Hardy, J. Quay*, R. Ader, and S. Livnat*. Dept of Neurobio./Anatomy and Dept of Psychiatry, Univ. of Rochester Med. Ctr., Rochester, NY 14642.

Conspecific aggression is a naturally occuring environmental stressor. While certain behavioral responses to stressors are known to modify a variety of pathophysiological changes, little is known about the influence of conspecific aggression on the immune system.

In the present study, adult male mice of two inbred strains (C3H/HeJ and Balb/cByJ), housed individually for 6 or 10 wks, were paired daily for 10 min on the same side of a 17 x 9 x 8 in cage, which was divided in half by a wire mesh barrier. During 14 test sessions, behavioral interactions (e.g. dominance) were noted. Control groups included: mice paired across the barrier from each other (non-fighting) and "witness" mice paired across the barrier from a fighting pair. After 14 d the mice were sacrificed, and spleen and lymph nodes harvested. In vitro proliferative responses of lymphocytes to T or B cell - selective mitogens were examined and the activity of spleen natural killer (NK) cells was evaluated.

C3H mice showed more aggressive behavior than Balb/c mice. Strain differences for several behaviors were noted in dominant / submissive pairs (e.g. chasing and biting the submissive mouse was more frequent in C3H pairs). The immunological consequences of dominance was also more evident in C3H mice, in which dominant mice had a significantly elevated T cell mitogen response. Differences in T, B, and NK cell activities were not observed between any of the treatment groups in Balb/c mice.

Our results indicate that genetic factors and behavioral responses of dominant / submissive mice interact to modulate the impact of a natural form of stress on the function of T lymphocytes, which play multiple regulatory and effector roles in host defense.

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438.10 NATURAL KILLER CELL ACTIVITY IN SCHIZOPHRENIA. Z. Kronfol, M.P.N. Nair*, H. Soudah*, G. Rhodes-Reed*, R. Bharucha-Reid*, S.A. Schwartz* and V.M. Hawthorne*. Departments of Psychiatry, Epidemiology and Pediatrics, The University of Michigan Medical Center, Ann Arbor, MI 48109.

The central nervous system and the immune system are closely interrelated. Neural lesions and stress have been shown to interfere with immune regulation. Conversely, several immunological reactions have been associated with specific neurochemical and neurophysiological changes. The clinical implications of these findings have not been adequately investigated. We have earlier reported an impairment in mitogen-induced lymphocyte proliferation in patients with depressive illness. Natural killer (NK) cell activity is another important parameter of cellular immune function. NK activity has been reported to be decreased in several stressful situations such as bereavement and academic stress. NK activity has not been adequately studied in specific psychiatric disorders. We now report our preliminary findings on the NK activity of patients with schizophrenia.

Forty-nine hospitalized schizophrenic patients, all meeting DSM III criteria for schizophrenia, and 19 normal control volunteers participated in the study. NK cell activity was evaluated in vitro using a 4 hr ^{51}Cr release assay against K562 target cells at different effector:target (E:T) cell ratios. Blood samples from schizophrenic patients and normal controls were run simultaneously to control for day-to-day variation in the assay. Paired t-test analysis revealed a significant reduction ($p < .0005$) in the NK activity in the schizophrenic group. Furthermore, 14/49 (28%) of the schizophrenic patients showed >50% reduction of NK activity compared to their respective controls. Similar findings were seen across different E:T cell ratios.

These results indicate an impairment in NK cell activity in patients with schizophrenia. It is not clear at present whether these findings represent a fundamental neuroimmunological abnormality in schizophrenia or whether they reflect other factors such as infection, hospitalization or nutritional status. Research is presently underway to address some of these issues.

438.11 AUTOIMMUNE MRL MICE SELF-SELECT THERAPEUTIC DOSES OF CYCLOPHOSPHAMIDE. T. R. Schachtman,* J. Moynihan,* L.J. Grota, & R. Ader*. (Spon: E. Caine). Dept. of Psychiatry, University of Rochester School of Medicine & Dentistry, Rochester, NY 14642 USA

MRL-lpr/lpr mice develop lymphadenopathy and autoimmune disease between 12-20 wks of age and the congenic +/+ substrain shows no evidence of autoimmune disease at these ages. Immunosuppression during this time is therapeutic for lpr animals. Based on other behavioral (conditioning) data, we hypothesized that lpr mice, in contrast to +/+ mice, would adopt behaviors that result in immunosuppression. To test this hypothesis, we gave lpr and +/+ mice the opportunity to drink solutions containing cyclophosphamide, an immunosuppressive drug.

Water deprived lpr and +/+ mice were randomly assigned to treatment groups at 19-20 weeks of age. In the morning, a drinking bottle containing chocolate milk (Sealtest homogenized whole milk) and 0, .2, .4, or .8 mg of cyclophosphamide per ml was placed on the cage for 1 hr. Consumption of the mixture was estimated by weighing the bottle before and after the drinking period. Supplemental water was made available in the afternoon. At weekly intervals, the mice were palpated for lymphoproliferation, eye bled for serum anti-DNA antibody titer, and weighed.

In 2 replications, there was an inverse relationship between the amount consumed and the concentration of cyclophosphamide in the drinking solution. After 1 week of exposure, there were no differences between lpr and +/+ males irrespective of concentration. After 2 or more weeks of exposure, lpr animals consumed more cyclophosphamide than +/+ animals at low concentrations of cyclophosphamide (.2 & .4 mg/ml). This pattern of consumption occurred earlier in males than females.

The dose of cyclophosphamide ingested was positively correlated with the concentration of cyclophosphamide in the drinking solution up to a maximum at .4 mg/ml. Lymphoproliferation and anti-DNA antibody titer were not altered in animals exposed to chocolate milk alone. In animals exposed to cyclophosphamide, both lymphoproliferation and anti-DNA antibody titer were reduced as a function of both concentration of cyclophosphamide and weeks of exposure.

In contrast to congenic controls, MRL animals with autoimmune disease will voluntarily consume chocolate milk containing cyclophosphamide in an amount sufficient to attenuate their immunologic disease. These results implicate behavior in the modulation of immune state.

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438.12 AUTOIMMUNE DISEASE MODIFIES CYCLOPHOSPHAMIDE INDUCED TASTE AVERSION IN MRL MICE. L. J. Grota, R. Ader*, and N. Cohen*. Dept. of Psychiatry and Immunology, University of Rochester Medical Center, Rochester, NY 14642 USA

Taste Aversion in normal mice is induced by cyclophosphamide by producing gastrointestinal upset and immunosuppression. Cyclophosphamide is immuno-therapeutic for mice with active autoimmune disease. We hypothesized that therefore animals with autoimmune disease would show poorer cyclophosphamide induced taste aversion than animals that do not have autoimmune disease.

Water deprived autoimmune MRL lpr/lpr mice and age matched MRL +/+ mice without autoimmune disease were given chocolate milk to drink followed by the injection of 0, 25, 50, 100, or 200 mg cyclophosphamide/ kg body wt. A preference of 42% for chocolate milk relative to water (200 mg group) after three reinforced trials defined the criterion for aversion. Reinforced trials (3/wk) were given until an animal reached criterion or for 10 trials. Among the lpr/lpr animals, 47% reached criterion compared to 92% of the +/+ animals ($p<.01$) at 100 mg cyclophosphamide but there were no differences at the other doses. On the initial 3 preference tests lpr mice have poorer taste aversion than +/+ mice as the dose and number of trials increase.

Other lpr/lpr and +/+ mice were exposed to chocolate milk and 0, 50, 100, or 150 mg cyclophosphamide/ kg body wt before either had autoimmune disease. Analysis of variance of preference scores revealed interactions of Trials X Dose and Trials X Sex but no differences due to strain.

Additional 10 and 18 wk old MRL mice were presented with chocolate milk followed by the injection of 0 or 500 mg LiCl/ kg body wt. Analysis variance of scores from 3 preference tests revealed an interaction between Strain, Sex, Age, and Drug ($p<.05$). Overall, the data indicate that lpr/lpr mice can lean LiCl induced taste aversions as well as or better than +/+ mice.

Finally, 10 & 18 wk old MRL mice were tested for shock induced passive avoidance behavior. On an initial extinction trial, there were no differences due to strain nor to age; 65% of animals avoided the dark chamber. The rate of extinction was faster for 18 wk old compared to 10 wk old mice and for lpr/lpr compared to +/+ mice.

These data indicate that MRL mice with active autoimmune disease do not learn taste aversion induced by the immunosuppressive drug cyclophosphamide as well as MRL mice that do not have active autoimmune disease. Since there were no differences in taste aversion induced by LiCl nor in shock induced passive avoidance behavior, we conclude that the differences in taste aversion induced by cyclophosphamide were related to the therapeutic effects of the drug for MRL mice with active autoimmune disease. These data indicate that the immune status of an animal can modify its behavior.

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439.1 PROGESTERONE ACTS THROUGH A CENTRAL NERVOUS SYSTEM STEROID RECEPTOR MECHANISM TO STIMULATE RESPIRATION. D.A. Bayliss, D.E. Millhorn, E.A. Gallman, J.B. Dean and J.A. Cidlowski*. Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27514.

The steroid hormone progesterone is a putative ventilatory stimulant although neither its site nor mechanism of action have been elucidated. We investigated the possibility of a central effect of progesterone on respiration. Progesterone was administered intravenously or directly into the medulla oblongata of anesthetized and paralyzed male and female cats. Carotid sinus and vagus nerves were cut and end-tidal PCO_2 and temperature were kept constant with servocontrollers to nullify effects of known respiratory feedback mechanisms. Phrenic nerve activity was measured as an index of central respiratory output. Repeated doses of progesterone (0.1 to 2.0 µg/kg) given intravenously caused a sustained (>45 minute) elevation of phrenic nerve activity in both male and female cats. The mean increase in phrenic nerve activity was similar in both males and females; however, the female cats, which were presumably at different states of estrous, showed a more variable response than the males. Spinal transection at C_7/T_1 to eliminate potential visceral afferent input had no effect on the response. Pressure injections of progesterone (either 1.0 nmole or 0.9 pmoles) into the region of the nucleus tractus solitarii, an area known to be associated with respiration, caused a similar enhancement of phrenic nerve activity. In addition, progesterone administered via either route caused a substantial hypotension. The possible involvement of a specific steroid receptor in the central respiratory response was also examined. Identical intravenous doses of other classes of steroid hormones (17β-estradiol, testosterone, cortisol) did not elicit the same respiratory response. Furthermore, pretreatment with RU486, a progesterone receptor antagonist, blocked the facilitatory effect of progesterone. We conclude, therefore, that progesterone acts in the central nervous system through a specific steroid receptor mechanism to cause a long-lasting facilitation of respiration.

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439.2 IMMUNOCYTOCHEMICAL LOCALIZATION OF CARBONIC ANHYDRASE IN MEDULLARY REGIONS OF RESPIRATORY CHEMOSENSITIVITY. J. A. Neubauer*, H. M. Geller and N. H. Edelman*. UMDNJ-Robert Wood Johnson Medical School at Rutgers, Piscataway, N.J. 08854.

Carbonic anhydrase (CA) inhibition in peripherally chemodenervated cats significantly delays the dynamics of the respiratory response to CO_2. This suggests that central transduction of the CO_2 sensory signal requires CA. We have used immunocytochemical techniques to identify neurons in dissociated cultures of central chemosensitive regions of the ventral medulla which contain CA. Since previous studies have shown that CA is located in CNS oligodendrocytes and dorsal root ganglion neurons, dissociated cultures of optic nerve and dorsal root ganglia were also prepared. Finally, since hypothalamus contain several populations of neurons which subserve sensory functions, cultures of this region were also examined for the presence of CA. Dissociated cultures of these regions were prepared by enzymatic and mechanical treatment from tissue dissected from neonatal rat pups. After 5-7 days, cultures were fixed in 5% Acrolein in 0.1M PBS and permeabilized by treating with 0.25% Triton x 100. Prior to incubation with antisera, the fixed cultures were preincubated with a blocking buffer (normal goat serum) and then incubated with a primary antisera directed against carbonic anhydrase C (from Dr. P. Linser, C.V.W., Fla). Specific binding of CA was visualized using the avidin-biotin peroxidase technique with a DAB reaction product. We found that consistent with previous reports, oligodendrocytes cultured from optic nerve and the majority of neurons in dorsal root ganglia stained positive for CA. Within the ventral medulla and hypothalamus all nonneuronal cells stained lightly for CA. Only a small percentage of neurons cultured from the ventral medulla stain for CA while nearly half the hypothalamic neurons stain for CA. The large number of cultured hypothalamic neurons with CA may be related to the recently described CO_2 sensitivities of the hypothalamus. We conclude that there are neurons in the chemosensitive regions of the ventral medulla in culture which contain CA and speculate that only a small number of neurons are responsible for the CO_2 sensitivity of this region. (Supported by N.I.H. HL-33938).

439.3 SEROTONERGIC AND CHOLINERGIC INFLUENCE ON POSTERIOR CRICOARYTENOID MUSCLE FUNCTION. R. Lydic. Pulmonary Div., The Penn. State Univ., College of Med., Hershey, PA 17033.

Several lines of evidence suggest that serotonergic (5-HT) and cholinergic (ACh) brain stem neurons mediate some sleep-dependent changes in upper airway function. There is 5-HT input to the upper motoneurons of the posterior cricoarytenoid muscles (PCA) which abduct the larynx (Holtman, J.R. Fed.Proc., 46:1104, 1987), and 5-HT neurons in raphe obscurus contribute an excitatory input to phrenic motoneurons (Holtman et al J. Neurosci., 6:1185, 1986). Cellular studies have demonstrated that 5-HT neuron discharge ceases during REM sleep, at a time when cholinoceptive cells in the pontine reticular formation increase their discharge. To what extent may sleep-related changes in 5-HT or ACh systems mediate the sleep-dependent increases in airway resistance and decreases in electromyographic (EMG) activity of the PCA muscles?

Preliminary to investigations of intact, sleeping animals, the present study is using barbiturate anesthetized (35 mg/kg sodium pentobarbital) cats to examine the EMG response of the PCA and diaphragm (D) to electrical stimulation of the dorsal raphe nucleus (DRN) and to microinjection of carbachol into the medial pontine reticular formation (mPRF). Bipolar stimulating electrodes were aimed for the DRN (P=-1., L=0, H=-1.) where microstimulation (10-80 µA, 100-200 Hz) produced a 10% increase in peak integrated PCA activity and a 50% increase in similar measures of D activity. Stimulation could also alter respiratory cycle timing and produced a 50% reduction in the duration of expiration. The EMG effects were influenced by respiratory cycle phase and stimulation was most effective when administered during inspiration. Microinjections of carbachol (1, 5, 10, and 15 µg, each in 250 nl saline) were aimed for the mPRF (A=1.5, L=1.5, H=-5.0). A 40% slowing of respiratory frequency followed the 5 µg dose and after 10 µg there was increased EMG activity recorded from the PCA (18%) and D (10%). Following carbachol, the electroencephalogram (EEG) recorded from the suprasylvanian cortex showed fewer barbiturate spindles and more EEG synchrony.

The effects of DRN stimulation on the PCA are consistent with the excitatory 5-HT input to the D produced by stimulation of the medullary raphe. Carbachol did not produce EMG suppression of the D and PCA muscles. Anesthetics reduce hypoglossal nerve activity but the lumbar motor suppressive effect of carbachol persists following decerebration (Morales et al J.Neurophys., 57:1118, 1987). Future studies using unanesthetized animals will determine whether carbachol reduces upper airway motor tone in a manner similar to that seen during REM sleep.

439.4 GABAERGIC REGULATION OF RECURRENT LARYNGEAL AND PHRENIC NERVE ACTIVITIES IN THE CAT. K.A. King* and J.R. Holtman, Jr., Dept. of Pharmacology, Coll. of Med., U of KY, Lexington, KY 40536.

The purpose of this study was to investigate the involvement of the inhibitory neurotransmitter, GABA, in regulating recurrent laryngeal nerve (RLN) and phrenic nerve (PHR) motor outflows in the cat.

Recurrent laryngeal nerve and C5 PHR nerve activities were recorded in nine chloralose-urethane (50 mg/kg and 250 mg/kg) anesthetized, paralyzed, and artificially ventilated cats (2.2-3.3 kg). Neural discharge was quantitated by integration of each nerve's activity. Only the inspiratory activity in the RLN was able to be consistently quantitated. Respiratory rate was determined from recordings of integrated nerve activity. A femoral artery and vein were cannulated for recording of blood pressure and for peripheral drug administration, respectively. The GABA agonist muscimol was administered in a cumulative dose range of 0.1-3.2 µg into the cisterna magna. Following the last dose of muscimol, the GABA antagonist bicuculline was administered by the same route in three of the nine cats.

Muscimol produced a dose-related decrease in both RLN and PHR nerve activities following intracisternal administration. Responses were first seen following the 0.1 µg dose, with RLN activity falling to 77.6±8.1% of control and PHR activity to 88.0±5.1% of control. Onset of the decreases in RLN and PHR nerve activities were 61±23 sec and 32±8 sec, respectively. These times were not significantly different. Successive doses of muscimol produced further depression of RLN and PHR nerve activities with apnea occurring in both nerves. Apnea in each nerve was observed in 3 of 9 cats after 0.4 µg muscimol and was always produced in both RLN and PHR nerve activities following 2.4 µg muscimol. No significant changes in respiratory rate were observed in either nerve following muscimol. Administration of 100 µg bicuculline reversed the muscimol-induced apnea in both nerves. The reversal occurred within 86±22 sec for the RLN with activity in this nerve increasing to 253±77% of control. PHR activity returned in 77±8 sec and reached a level of 381±92% of control. There was no significant difference in the time to return of activity in each nerve.

These results suggest that both RLN and PHR nerve activities are regulated by GABAergic neurons. Similarities in onset time of muscimol-induced depression of each nerve's activity as well as the similar time of reversal of this depression by bicuculline are consistent with an action of these drugs at a common site. A possible site of action is the GABAergic system at the ventral surface of the medulla. (Supported by HL 36050 to J.R.H.).

439.5 KAINIC ACID (4.7 mM) APPLIED TO THE ROSTRAL VENTROLATERAL MEDULLA INHIBITS VENTILATORY CO_2 SENSITIVITY AND DECREASES BLOOD PRESSURE. E. Nattie* and W. St. John* (SPON. F. McCann). Dartmouth Medical School, Hanover, N.W. 03756.

To ask if neuronal cell bodies are involved in the ventilatory chemosensitive responses localized to the ventrolateral surface of the medulla, kainic acid (KA) was applied by pledget for 1-2 min to the rostral, intermediate, or caudal chemosensitive regions. The experiments were performed in chloralose-urethane anesthetized, paralyzed, vagotomized, glomectomized and servo-ventilated cats. Integrated phrenic nerve activity and blood pressure were measured as outputs. Application to the rostral area immediately and significantly increased both blood pressure and phrenic output. Both then decreased with blood pressure falling to levels below control in 8 of 9 cats by 90 min and with apnea ensueing in 6 cats by 40 min. In 5 cats in which CO_2 responses could be evaluated, KA significantly decreased the slope (-97+/-15%) and the maximum value (-52+/-18%) of the phrenic activity. Application to the intermediate area had no significant effect on blood pressure or phrenic activity although there was a tendency for phrenic activity to be increased. The CO_2 response slope was unaffected and the maximum value was increased significantly 1-2 h after KA treatment. Application to the caudal area slowly increased blood pressure slightly (NS) and by 90 min blood pressure decreased significantly. Integrated phrenic responses were variable. With CO_2 stimulation there was no significant effect on the phrenic response slope. The maximum value was increased significantly immediately but not later after KA treatment. These data indicate the existence of neuronal cell bodies near the surface of the rostral chemosensitive area of the ventrolateral medulla that in response to KA application result in excitation then inhibition of phrenic nerve activity and blood pressure and result in inhibition of ventilatory CO_2 sensitivity. (Supported by HL 28066 and HL 20574.)

439.6 POSTNATAL DEVELOPMENT OF PHRENIC MOTONEURONS IN THE CAT. W.E. Cameron, E.A. Volk*, B.S. Brozanski* and R.D. Guthrie*. Depts. of Neurobiology and Pediatrics, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

The discharge characteristics of phrenic motoneurons are strongly influenced by synaptic connections that are established after birth. The surface area available for receipt of these new synaptic connections is determined by the rate of growth of the cell membrane. We have set out to describe the development of phrenic motoneurons during the first postnatal months when these neural elements controlling the diaphragm play such a critical role in sustaining ventilation of the newborn.

Kittens in three age groups (1-2, 4-5 and 7-8 weeks) were selected for study. The C5 root of the phrenic nerve was exposed and sectioned during aseptic surgery under halothane anesthesia. The central end of this root was soaked in a 40% HRP solution for 30-60 minutes. After a 48-hour survival time, the animal was deeply anesthetized and transcardially perfused. The cervical spinal cord was removed and cut in 50 micron-thick horizontal sections and reacted with TMB. The outline of cell body and proximal dendrites of heavily labeled motoneurons were reconstructed using a 100X oil objective. The major and minor axes, perimeter (p) and cross-sectional area (A) were measured from these drawings using a digitizing tablet. The number of primary dendrites was recorded. In addition to the morphological parameters measured, average diameter, form factor, cell surface area and volume were calculated.

Fifty cells from each animal (two kittens per age group) were analyzed. The mean values (standard error) for the different age groups are summarized below.

Age group (weeks)	1-2	4-5	7-8
Ave. diameter	28.3 (.5)	34.1 (.4)	37.9 (.5)
Surf. area (μm^2)	2288 (66)	3274 (82)	3915 (94)
Volume (μm^3)	10302 (436)	17434 (634)	22459 (832)
#1° dendrites	6.1 (.1)	6.0 (.1)	5.8 (.1)
Form factor ($4\pi A/p^2$)	.94 (.004)	.94 (.004)	.92 (.005)

With the exception of the number of primary dendrites between all age groups and the form factor between the first and second age group, all other values differed significantly ($p<.01$) between age groups. Over the age ranges studied, the surface area has almost doubled while the number of primary dendrites has remained unchanged.

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439.7 **POSTNATAL MATURATION OF ELECTROPHYSIOLOGIC PROPERTIES OF NEURONS IN THE VENTRAL REGION OF THE NUCLEUS TRACTUS SOLITARIUS IN THE RAT.** G.G. Haddad and P.A. Getting. Dept. of Physiology and Biophysics, Univ. of Iowa, Iowa City, IA 52242.

Previous studies have shown that biochemical and morphologic changes occur with maturation postnatally in the brainstem of the rat. However, little is known about the electrophysiologic properties of neurons in the brainstem, particularly those involved with respiration. Since the inherent electrophysiologic properties of individual cells in a network can markedly influence the final output of that network, we examined the postnatal maturation in these properties of premotor neurons in the ventral region of the Nucleus Tractus Solitarius (v-NTS), an area considered to be important in shaping phrenic motor output. Intracellular recordings and current clamp techniques were applied to transverse brain slices obtained from a region near the obex (±0.7 mm) of adult Sprague-Dawley rats and rat pups (age = 3-10 d). Forty two v-NTS cells were studied in adult rats. Based on electrophysiologic criteria, these cells could be separated into 2 distinct groups. Type A cells (85% of total) exhibited a slow rate of firing at rest (1-2 spikes/sec) and marked spike frequency adaptation (SFA) with depolarization. Most type A cells (>80%) had an SFA ratio (steady state/peak firing frequency) of <0.5 at current intensities ≥0.4 nA. Type A cells also displayed delayed excitation (DE) which was manifested as a prolonged delay (up to 700 msec) before the onset of firing following a hyperpolarizing prepulse. Type B cells had a much higher rate of firing (10-20 spikes/sec) at rest, showed SFA but no DE. Depolarizing-after-potentials and postinhibitory rebound were also seen with this cell type. In contrast to adult cells, newborn v-NTS neurons (n=15) belonged to a single group which resembled neither type A nor type B of the adult rat. They showed significantly less SFA (80% of cells had an SFA ratio of >0.5) (Wilcoxon Rank Sum test), little or no DE, no depolarizing-after-potentials or postinhibitory rebound. Using intracellular staining techniques (Lucifer Yellow), we also found that substantial increase in arborization of processes and increase in cell soma size were associated with the maturational changes in the electrophysiologic properties. We conclude that in the newborn rat pup, v-NTS neurons do not possess the electrophysiologic properties of adult cells and must acquire them postnatally with maturation. Since the repetitive firing patterns of v-NTS cells are reflections of underlying membrane ionic mechanisms (Dekin and Getting, J. Neurophysiol., in press), differences between newborn and adult neurons probably indicate differences in specific ionic currents. The maturational differences in the firing patterns may have important implications on the integration of afferent synaptic input and on the shaping of respiratory motor output.

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439.8 **RESPIRATORY-LIKE ACTIVITY RECORDED FROM NUCLEUS TRACTUS SOLITARIUS CELLS IN SLICES FROM THE MEDULLA OBLONGATA OF THE RAT.** **J.B. Dean and D.E. Millhorn. Dept. of Physiology and Curriculum for Neurobiology, Univ. N. Carolina, Chapel Hill, NC 27514.**

It is well known that neuronal elements within the medulla oblongata are responsible for the generation of respiratory rhythm and for central chemosensitivity to CO_2 and H^+. Nevertheless, relatively little is known about either the anatomical substrate or mechanism of these important respiratory functions. The present study was done to determine in vitro if single cells in the nucleus tractus solitarius (NTS) show rhythmical activity or chemosensitive-like activity to CO_2/H^+. We have made single-unit recordings in the NTS using coronal medullary slices (400-450um) prepared from male Spraque-Dawley rats. Medullary slices were perfused continuously with nutrient medium (36-37°C) while maintained in an interface chamber. A warmed humidified control gas mixture of 5% CO_2 in O_2 was passed over the slices and bubbled through the medium. Under control conditions, many neurons fired at <5 Hz with a steady, continuous pattern; other neurons displayed a slow rhythmicity of 0.5-3 cycles/min. After isolating a spontaneously firing neuron for 2-3 mins, the gas was changed to 7 or 10% CO_2, balance O_2, for 2-5 mins. Thus far, chemosensitive-like activity has been found in 20-25% of the NTS neurons tested; excitation was the predominate effect. In one case, increased CO_2 induced a rhythmical pattern (2-4 cycles/min) in a neuron that had shown little activity. These results suggest that some NTS neurons show chemosensitive-like activity, supporting an earlier study (Brain. Res., 271:349, 1983). Moreover, NTS neuronal rhythmicity is retained in the tissue slice, but at a slower rate than in vivo. It had been suggested (Brain Res., 280:155, 1983; Science, 229:67, 1985) that NTS neuronal rhythmicity in vitro had to be pharmacologically induced. The slower rhythms reported here could be attributed to the removal of excitatory synapses normally impinging on these neurons in vivo. Studies are now underway using a high Mg^{++}/low Ca^{++} synaptic blocking medium to determine if chemosensitive-like activity and rhythmicity are inherent or synaptically derived properties of NTS neurons. **This study was supported by USPHS Grant HL33831 and NINCDS Training Grant 5-T32-NSO7166-08.**

439.9 **TRIPHASIC PATTERN OF MEMBRANE POTENTIAL IN RESPIRATORY NEURONS OF NEWBORN PIGLETS.** E.E. Lawson*, D.W. Richter*, D.L. Brown, and D. Ballantyne*. Dept of Pediatrics, Univ of N Carolina at Chapel Hill, and I. Physiologisches Institut der Universitaet Heidelberg, FRG.

The objective of the present study was to determine the pattern of phasic activity of individual respiratory related neurons and to determine whether synaptic inputs affect these patterns in newborns. Respiratory neurons were recorded in 12 piglets, 2-13 days of age. The piglets were anesthetized (pentobarbital), intubated, paralyzed, ventilated, and mounted in a rigid frame. The dorsal medulla was exposed by occipital craniotomy. Phrenic nerve activity was used as the index of respiratory phase. The vagus nerves were cut bilaterally and one was placed upon a bipolar stimulating electrode (0.8-5V, 0.05 or 0.5ms pulses). An array of concentric bipolar electrodes was placed into the C2 spinal cord to test for antidromic activation of bulbospinal neurons (5-20V, 0.05ms pulses). Intracellular recordings of neurons were obtained using glass microelectrodes 20-60 megOhms resistance containing 3MKCl or 2M Kacetate. Reversal of cyclic membrane potential patterns due to Cl^- or negative current injection was taken as evidence of inhibitory post-synaptic (ipsp) activity. Respiratory neurons were identified in two caudal medullary regions with stereotactic coordinates similar to the dorsal and ventral respiratory groups (DRG and VRG) of the adult cat. Neurons were classified according to the respiratory phase in which depolarization occurred. In one animal, 2 nonbulbospinal DRG inspiratory units were recorded both of which received excitatory post-synaptic potentials following ipsilateral vagus stimulation. Respiratory neurons were recorded in the lateral medulla (eg the VRG) of the remaining 11 piglets. Thirtyone inspiratory neurons (7 bulbospinal), 13 postinspiratory neurons (6 vagal), and 27 expiratory neurons (13 bulbospinal) were recorded (initial membrane potential >-35mV) at least for enough time to allow respiratory phase verification. In contrast to the usual two phase pattern of phrenic activity (eg ramp inspiration and expiratory silence), the neuronal pattern consisted of phases of hyperpolarization, depolarization, and apparent disfacilitation. For each of the three cell types the expiratory phase contained two distinct periods of membrane potential. Reversal of ipsps during the postinspiratory or later expiratory phases was demonstrated in inspiratory cells. In contrast reversal of ipsps during the inspiratory phase occurred in postinspiratory and expiratory cells. The triphasic pattern of membrane activity in the respiratory neurons was similar to that of adult cats (Richter, J Exp Biol 100:93, 1982). These data, from newborns, support the three phase theory of respiratory cycle control and demonstrate that inhibitory synaptic inputs dominate certain phases. (Support from NIH HL34919 and HD000475, Alexander von Humboldt Stiftung, and DFG)

439.10 INHIBITORY INFLUENCES ON EXPIRATORY NEURONS IN THE CAUDAL VENTRAL RESPIRATORY GROUP (cVRG) AS REVEALED BY INTRACELLULAR RECORDINGS. W.R. See, M.I. Cohen, R. Barnhardt* and C.N. Christakos*. Dept. of Physiology, Albert Einstein Col. of Med., Bronx, NY 10461.

In unanesthetized, decerebrate, paralyzed cats ventilated with a cycle-triggered pump, simultaneous recordings were taken of phrenic (PHR) and recurrent laryngeal (RL) nerve activity and of the membrane potentials (MP) [-40 to -60 mV at the time of largest hyperpolarization (HP)] of 12 augmenting expiratory (E) neurons in the cVRG (2.5-3.7 mm caudal of the obex, 3.0-3.5 mm lateral, 2.1-3.2 mm dorsal to the ventral medullary surface). The effect of pulmonary afferent (PA) inputs was assessed by withholding inflation during an inspiratory (I) phase and comparing respiratory cycle triggered histograms of the average activities in an equal number of control (inflation) and test (no-inflation) cycles.

The I-onset was accompanied by a rapid HP of 5-10 mV and cessation of neuron discharge. The HP in I had two patterns: a) approximately constant level, reversing to a steep depolarization (DP) shortly before the I-offswitch (8 cases); b) a gradual DP ramp throughout I (4 cases). The E-phase MP trajectory consisted of an initial rapid DP followed by a ramp DP which usually continued throughout E.

During no-inflation, the HP level in I was increased, and the DP ramp diminished, leading in most cases to a delayed onset of E-discharge. This was the case even when inspiratory time was not lengthened and peak PHR amplitude was decreased in comparison with inflation I phases. After no-inflation, the initial DP during E was often followed by an HP wave of variable duration and intensity, accompanied by a transient inhibition of neuron discharge. These early-E HP components always showed a close association in time with an increase of early-E RL activity, but only in some cases were they associated with a burst of post-inspiratory PHR activity.

The results show: (1) HP of cVRG E-neurons during I is influenced by PA feedback. (2) Increased HP during functional elimination of PA input is not necessarily related to increased activity of augmenting I-neurons since it appears also in cases with reduced PHR activity. This increase of HP may be due to greater inhibition by neurons with a plateau or decrementing firing pattern similar to that of hypoglossal or RL, since discharge of such neurons is increased during no-inflation. (3) Transient early-E HP may be related to inhibition by early-E decrementing neurons (with activity patterns and responses to no-inflation similar to those of early-E laryngeal motoneurons), whose discharge is greater following no-inflation (greater initial discharge and slower decay) as has been shown in medullary recordings.

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439.11 FUNCTIONAL ASSOCIATIONS OF VENTRAL RESPIRATORY GROUP NEURONS WITH MIDLINE AND CONTRALATERAL RESPIRATORY-MODULATED BRAIN STEM NEURONS. B.G. Lindsey, L.S. Segers* and R. Shannon. Dept. of Physiol. and Biophysics, Univ. South Florida Med. Ctr., Tampa, FL 33612.

Cross-correlation analysis of simultaneously recorded neurons located in the same side of the medulla suggest roles for at least two synaptic processes in the development and termination of each phase of breathing (1,2). Recordings made following midsagittal section of the medulla and brain stem transections at the pontomedullary and C1 levels reveal rhythmic activity in each half of the medulla (3). The two halves discharge asychronously. Mechanisms that coordinate the bilaterally distributed rhythm generating processes are not well understood. We now report evidence for interactions between respiratory-related neurons located in opposite sides of the medulla and between cells located near the midline and those in lateral groups. Data were obtained from 31 anesthetized (Dial), paralyzed, bilaterally vagotomized, artificially ventilated cats. Recording and analytical methods have been described (1,2). Electrodes were placed in two or more of the following locations in each cat: left rostral ventral respiratory group (VRG) near retrofacial n., right rostral VRG, left caudal VRG (near n. retroambigualis, n. ambiguus), n. raphe obscurus, n. raphe pallidus, and the midline at the pontomedullary border (4). Results, expressed as ratios of correlations/# pairs analyzed, are summarized in the table. The 901 cells pairs studied are grouped according to locations of constituent cells (rows) and phase of maximum discharge of cells (I-inspiration, E-expiration; columns). Cells with complex or little or no respiratory modulation are designated Others. Short time-scale correlations were indicated by central peaks or troughs, offset peaks or troughs and, most commonly, multiple low amplitude peaks in correlograms.

	I-I	E-E	E-I	I-Other	E-Other
rVRG-contra. rVRG*	16/68	13/130	15/153	3/31	1/22
cVRG-contra. rVRG	6/69	6/37	4/52	2/13	1/11
rVRG-midline	2/10	9/35	3/50	5/55	6/67
cVRG-midline	1/9	1/8	1/3	3/42	0/9

*r-rostral c-caudal

The results indicate functional interactions between spatially distributed neurons appropriate for roles in both coordinating and contributing to bilaterally distributed rhythm generating processes and for shaping the respiratory-modulated discharge patterns of midline neurons.

References: 1,2. J. Neurophysiol. 57:1078-1100; 1101-1117, 1987. 3. J. Appl. Physiol. 55:433-439, 1983. 4. Respir. Physiol. 61:153-166, 1985. Supported by NIH NS19814.

439.12 PARABRACHIAL SUBNUCLEI IN RESPIRATORY CONTROL. H.R. Holmes, H. Herbert and C.B. Saper. Dept. Pharm. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

The parabrachial nucleus (PB), the classical "pneumotaxic center," is recognized as necessary for maintaining normal respiratory rhythm. Electrical stimulation of PB areas elicits variable effects on respiration, but no clear picture of the functional organization of PB has emerged. In view of our findings on the cytoarchitecture and connections of PB (Br Res Rev 1984; 7:229), we have re-examined the functional organization of PB subnuclei for respiratory control.

Respiratory airflow and blood pressure were monitored in α-chloralose anesthetized, spontaneously breathing rats during electrical stimulation (15 sec trains of 50 Hz, 0.25 msec pulses) of PB subnuclei using glass microelectrodes filled with 2 M NaCl and pontamine sky blue to mark each electrode track. Respiratory responses, threshold currents, and electrode coordinates were recorded for each stimulation site every 100 μm in tracks spaced 200 μm apart. Following each experiment, the brain was fixed in 10% formalin, sectioned at 50 μm and stained with thionin. Maps of PB subnuclei and electrode tracks were made by drawing the sections using a projection microscope, and coordinates of the effective stimulation sites were plotted on them.

Changes in tidal volume and respiratory rate were obtained at minimal current thresholds of 3-5 μA. Hyperpnea was produced by stimulation of the external or the central lateral subnucleus. Hypopnea or apnea was produced by stimulation of the Kolliker-Fuse subnucleus. Additional respiratory responses were obtained by stimulation of extreme, dorsal or central lateral subnuclei, or the medial subnucleus. Some of these responses seemed to depend on the rostral-caudal level which was stimulated.

These results demonstrate that opposite respiratory effects can be achieved by stimulating different subnuclei of the PB which have different projections. By combining microstimulation with anterograde neuronal tracing, it should be possible to identify specific cell groups and pathways that participate in discrete respiratory pattern control.

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H.H. is a DAAD/NATO Research Training Fellow.

439.13 EFFECTS OF ROSTRAL PONTINE STIMULATION ON MEMBRANE POTENTIAL OF EXPIRATORY MEDULLARY NEURONS. M. Fournier, D.W.Richter and J.L. Feldman. Systems Neurobiology Lab., Kinesiology Dept., UCLA, Los Angeles, CA 90024-1568 and I. Physiologisches Inst,. Universität Heidelberg, Heidelberg, FRG.

Low intensity electrical stimulation of the rostral pontine respiratory group (PRG) produces profound alterations in respiratory pattern; we studied the effects of PRG stimulation on the membrane potential (Vm) of expiratory-related (E) neurons of the ventral respiratory group (VRG). Experiments were performed on anesthetized, paralyzed, vagotomized and mechanically ventilated cats. Stimulation was made with a tungsten electrode (~2 MΩ) inserted into the PRG. Intracellular recordings (3 M KCl) were made from ipsilateral VRG E cells. Among 16 E cells with a Vm < -40 mV, 10 were bulbospinal, 3 laryngeal and 3 were not antidromically activated. Only 4 VRG E cells could be activated synaptically by single pulse stimulation of the PRG (latencies: 12-20 ms). Within the range of PRG stimulation used (3-30 μA, 100 Hz, 0.2 ms), effects on Vm were intensity-related only when stimuli were delivered during early inspiration (I) and early E. Low intensity stimulation of PRG during early I, which depressed phrenic nerve activity, transiently depolarized E neurons. With increased stimulus intensity associated with inspiratory phase termination, Vm exhibited a post-inspiratory shift characterized by a steep depolarization leading to E discharges. With stimulation during late I, Vm exhibited a post-inspiratory shift independent of stimulus intensity. PRG stimulation during post-inspiration, which abolished post-inspiratory activity of the phrenic nerve, increased the rate of depolarization in E neurons, leading to earlier initiation of the E spike burst. PRG stimulation at low intensity during mid-E caused little or no change in Vm, but with increased intensity, induced a transient hyperpolarization. Stimulation during late E, terminating the E phase, was marked by a sharp hyperpolarization of Vm, suggesting an enhanced early I inhibition. That the intensity-dependent effects occurred only during early I and early E suggest that PRG influences on respiratory pattern are mediated or modulated by early I and post-inspiratory cells. Supported by NIH Grant HL 37941.

439.14 CHANGES IN FREQUENCY CONTENT OF INSPIRATORY NERVE AND NEURON ACTIVITY DURING INSPIRATION. C.N. Christakos*, M.I. Cohen and W.R. See. Physiol. Dept., A. Einstein Coll. Med., Bronx, NY 10461

The spectral composition of inspiratory (I) nerve activity [phrenic (PHR) and recurrent laryngeal (RL)], and specifically the strength of high-frequency oscillations (HFOs, range 50-100 Hz) and medium-frequency oscillations (MFOs, range 20-50 Hz), is known to vary during I (Richardson & Mitchell, Brain Res. 233, 317-336, 1982). It has also been reported that MFOs are not present in early-I PHR activity and it has been suggested that their occurrence later in I is due to activity of late recruited PHR motoneurons (MNs) (Webber, Soc. Neurosci. Abstr. 12, 305, 1986). Since medullary (DRG and VRG) I neurons and also cervical I neurons show HFO coherent to the PHR HFO (Christakos et al., Fed. Proc. 46, 1102, 1987), their behaviour at various stages of I is of interest. We therefore performed spectral analysis on spike trains of DRG, VRG and cervical I neurons, as well as on activity of PHR and in some cases RL nerves, all obtained from decerebrate cats that were paralyzed and ventilated with a cycle-triggered pump. To study the changes during I, the activities were gated to different parts of I (e.g., halves), including the start of I (initial stage lasting 50-100 ms that often shows a PHR burst).

Our data showed that: (1) PHR and RL I activity can exhibit HFO or MFO or both at all stages of I, except for the start of I where there is no MFO in PHR. MFOs, which are not coherent between PHR and RL, tend to increase in amplitude throughout the I phase. The HFO grows or stays the same for up to two thirds of I and becomes weaker or even vanishes in late I. The PHR-RL coherence of the HFO changes in a similar way. (2) DRG and cervical I neurons show no MFO at any stage of I. Late in I, some VRG neurons show a transition from the HFO to a somewhat lower-frequency rhythm which, unlike the HFO, does not correspond to any prominent frequency components of PHR and is not coherent to PHR. The HFO of medullary and cervical neurons changes during I in a way similar to the PHR HFO, and the same holds for their coherences to PHR. When the PHR HFO vanishes in late I, the unit HFO and the coherence do the same.

These observations indicate that MFOs are specific to different MN pools and in PHR are unlikely to originate from premotor networks in the manner the HFO does. MFOs might arise from subsets of MNs having rhythms in the MFO range, and their changes during I could then reflect changes in the distribution of the properties of the active MNs. In contrast, the HFO is a general property of the system and is likely to arise in a common I pattern generator. The changes of HFO during I may provide information on the time course of interactions of I neuron populations.

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439.15 SYNCHRONIZATION OF RESPIRATORY, STEP, AND WINGBEAT CYCLES DURING LOCOMOTION IN THE CANADA GOOSE. G.D. Funk*, W.K. Milsom* and J.D. Steeves. (SPON: D. Schwarz). Dept. of Zoology, University of British Columbia (UBC), Vancouver, B.C., V6T 2A9.

Cross correlation analyses in mammals have demonstrated that breathing and step cycles are synchronized to some degree during locomotion, although the mechanisms for this coupling remain unclear. We examined the importance of telencephalic structures to the synchronization of locomotor and respiratory cycles in birds, as well as the effects of changes in the mode of locomotion on this synchronization. Decerebrate Canada geese were induced to walk (3-5 min) or wing flap (up to 30 sec) by electrical stimulation (25-100uA,60Hz) of the dorsolateral parvocellular reticular formation (PRF) or the ventromedial gigantocellular reticular formation (Rgc).

Minute ventilation ($\dot{V}_E$), the product of ventilation frequency (f) and tidal volume (V_T) were monitored via pneumotachography. Stepping and wing beat motor patterns were recorded with electromyographic techniques. Breathing and locomotor frequencies were measured over 1-10 sec intervals and the degree of synchronization was recorded as a function of elapsed time into exercise.

During walking, ratios of 2 or 4 steps per breath were most commonly found and this synchronization took 40-60 seconds to develop. Similarly, when the treadmill speed was altered, the breathing pattern readjusted to match the new stepping frequency within 20-40 seconds. In contrast, during wing flapping, respiratory and wing beat frequency became synchronized almost immediately which may reflect an obligatory mechanical coupling of the two muscle systems. The onset of flight movements was associated with a drastic change in breathing pattern whereby a 5-10 fold decrease in V_T was associated with a 10-25 fold increase in f resulting in the maintenance of a relatively constant level of alveolar ventilation.

These data suggest that the mechanisms responsible for establishing the synchronization between respiratory and locomotor cycles do not require forebrain structures and that these mechanisms differ for brachial and pelvic girdles.

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439.16 POST-HYPOXIC FACILITATION AND INHIBITION OF RESPIRATION IN GLOMECTOMIZED CATS. E.A. Gallman and D.E. Millhorn, Department of Physiology, University of North Carolina, Chapel Hill, North Carolina 27514

The respiratory response following hypoxia was studied in peripheral chemo-denervated cats with intact brains and following decerebration at two levels. Transection of the neuraxis rostral to the mesencephalon was termed high decerebration. Mid-collicular decerebration, extending to the rostral edge of the pons ventrally, was termed low decerebration. Cats were anaesthetized, paralysed and ventilated with 100% O_2. End-expired PCO_2 and temperature were servo-controlled. Central respiratory drive was determined as the product of peak integrated phrenic nerve activity and respiratory rate. Hypoxia was induced by ventilating cats for 10 min at reduced inspired oxygen levels (F_IO_2 = 0.06 to 0.15). The degree of resultant hypoxemia was determined from a blood sample drawn at the end of the 10 min hypoxia. During hypoxia respiration was depressed, often to the point of apnea. Of interest to us, however, was the response following the return to ventilation with 100% O_2. In cats with intact brains, the most severe hypoxia (P_aO_2 <25 torr) led to an inhibition of respiration lasting up to one hour following the end of the hypoxic episode. In contrast, mild hypoxia (P_aO_2 > 35 torr) was followed by a long-lasting facilitation of respiration. Ablation studies further extended these observations. The high decerebrate cats, i.e., those with mesencephalon intact, exhibited inhibition following even mild hypoxia. The low decerebrate cats showed neither inhibition nor facilitation following hypoxia. Additional experiments were done involving discrete stimulation of sites in the mesencephalon. It was possible to reduce phrenic activity both with electrical stimulation and with pressure injections of excitatory amino acids in the vicinity of the red nucleus. We propose that acute hypoxia activates two centrally mediated, long-lasting respiratory responses simultaneously. The inhibitory mechanism requires the presence of the mesencephalon and may involve the red nucleus. Higher brain structures, notably the diencephalon, are necessary for facilitation. The respiratory response during and following hypoxia is influenced by both mechanisms to varying degree.

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439.17 YAWNING: NO EFFECT OF CO_2, O_2 AND EXERCISE. R. R. Provine, B. C. Tate*, and L. L. Geldmacher*. Dept. Psychol., Univ. of Md. Baltimore County, Catonsville, MD 21228.

Using human college age subjects, the present study tested the commonly cited but previously untested hypothesis that yawning is facilitated by higher than normal levels of CO_2 or lower than normal levels of O_2 in the blood by comparing the effect on yawning of breathing 100% O_2, and gas mixtures with higher than normal levels of CO_2 (3% or 5%) with compressed air, the control condition. If yawning is a response to heightened blood CO_2, the CO_2 mixtures should increase yawning rate and/or duration. If low blood O_2 produced yawning, breathing 100% O_2 should inhibit yawning. The CO_2/O_2 hypothesis was rejected because breathing neither pure O_2 nor gases high in CO_2 had a significant effect on yawning although both increased breathing rate. A second study found that exercise sufficient to double breathing rate had no effect on yawning. The two studies suggest that yawning does not serve a primary respiratory function and that yawning and breathing are triggered by different internal states and are controlled by separate mechanisms.

Further evidence that yawning is not primarily a respiratory act comes from an analysis of the routes of inhalation and exhalation during yawning and the relation between yawn duration and inter-yawn interval (also see Provine, R. R., Ethology, 72, 109-122). Inhalation and exhalation occur primarily through the mouth and this basic pattern is highly inflexible. It is very difficult, if not impossible, for most people to perform a satisfying yawn with their mouth taped shut even though they are free to respire through their nose and move their jaw to a limited extent within their closed mouth. Yawning does not have the degree of behavioral freedom characteristic of normal breathing. However, oral inhalation by itself is insufficient to produce a satisfactory yawn if the jaw is not free to move. Subjects attempting to yawn with clenched teeth often reported the unpleasant sensation of being stuck in mid-yawn and being unable to perform a satisfying yawn although they could inhale and exhale through their teeth. Thus, the yawn is not simply a deep breath. The finding that performers of short yawns do not compensate by yawning more frequently and vice-versa is also inconsistent with the yawn as a respiratory event.

439.18 EFFECT OF HIGH PRESSURE ON SPONTANEOUSLY ACTIVE RESPIRATORY DRIVE IN ISOLATED MEDULLA OF NEWBORN RATS. A. Tarasiuk* and Y. Grossman* Unit of Physiology, Faculty of Health Sciences, Corob Center for Medical Sciences, Ben Gurion University of the Negev, Beer Sheva 84105, Israel. (SPON: K.R. Courtney)

High pressure, which induces hyperexcitability and convulsions in intact animals (High Pressure Nervous Syndrome), slows respiratory rate and increases lung tidal volume. In humans, these effects are associated with dispnea. The present experiments were designed to distinguish the central effects of pressure from the direct effects of compressed gas on lung mechanics. The medulla and spinal cord of newborn rats were isolated, placed in a pressure chamber and constantly superfused with oxygenated (95% O_2; 5% CO_2) bicarbonate-buffered (pH 7.3) Ringer solution at 27°C. Spontaneous rhythmic activity indicative of "respiratory drive" was recorded extracellulary from the cut C-4 and C-5 ventral roots, which give rise to the phrenic nerve. Changes in rhythmic activity were induced by extracellular stimulation of the fifth cranial nerve. After control measurements at 1 atm, the pressure was raised to 100 atm with compressed helium. Exposure to high pressure caused a reduction in the rate of spontaneous activity by 50%, decreased the amplitude of each spontaneous burst of activity by 10-20%, and increased its time integral by 30%. Brief (10-30 sec) stimulation of the fifth nerve at 10HZ and 1 atm transiently (30-40 sec) increased the frequency of respiratory drive and decreased the durations of individual bursts of activity. High pressure prolonged this period of increased frequency to minutes, without significantly affecting the durations of individual activity bursts. Stimulation of the fifth nerve at lower frequencies (1-5 Hz) for 1 min had no effect at 1 atm. However, when delivered under high pressure, this stimulation induced an entirely different pattern of spontaneous activity in the ventral roots. These results demonstrate for the first time a direct effect of pressure on the neural centers which control respiration. Although spontaneous activity is suppressed under pressure at steady state, the system is nevertheless rendered hyperexcitable to repetitive sensory stimulation, and net respiratory drive may therefore rise to aboove the control level.

PAIN MODULATION V

440.1 COCAINE: MECHANISMS OF CNS ANALGESIC ACTION IN THE RAT. Yu Lin*, T.J. Morrow & K.L. Casey, Depts of Neurology & Physiology, Univ. of Michigan and Neurology Research Laboratories, VA Medical Center, Ann Arbor, MI 48105

Recently, we showed in the rat that systemically administered cocaine HCl (25mg/kg, i.p.) acts as a rapid onset, non-sedative, centrally acting analgesic as determined by both the standard tail flick (n=30) and formalin (n=24) tests. Reduction of pain behavior in the formalin test was immediate (within 5 minutes), complete (pain score drop from 1.5-2.0 to 0.5-0.2) and sustained (lasting more than 1 hour). Accordingly we sought to determine the mechanism(s) involved in cocaine analgesia. Using the formalin test to access changes in pain behavior, we attempted to block the analgesic effects of cocaine using a known opiate (naloxone, 1 mg/kg i.p.) and dopamine (chlorpromazine, 3 mg/kg, i.p.) antagonists. We also compared the analgesic effect of cocaine with those produced by morphine (6 mg/kg, i.p.). We found that cocaine HCl, given intraperitoneally, produced a profound behavioral analgesia in a dose-dependent fashion, such that 20 or 25 mg/kg caused complete loss of pain responsiveness, while 15 and 10 mg/kg doses showed progressively weaker and shorter duration effects. Five mg/kg doses of cocaine had no analgesic action. Cocaine also failed to produce analgesia when administered by either intramuscular or subcutaneous injection. Naloxone, which reversed the effect of morphine, had no effect on the pain behavior of controls or cocaine treated animals (n=36). In contrast, chlorpromazine partially (n=9) or completely (n=3) blocked the analgesia produced by cocaine when given concurrent with (n=6) or 30 minutes prior to (n=6) cocaine. In 9 rats showing partial attenuation of cocaine analgesia, the onset was more gradual and the time course significantly shortened. Chlorpromazine had no effect on the pain behavior of control animals or on the analgesia produced by morphine. In conclusion, these data clearly show: (1) that in the rat, systemically administered cocaine HCl has a profound and dose-dependent central analgesic action and (2) that this analgesia is mediated by non-opiate mechanisms.

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440.2 COCAINE; NEUROPHYSIOLOGICAL EFFECTS ON BULBORETICULAR PROJECTION NEURONS. C. R. Belczynski*, A. Pertovaara, T. J. Morrow and K. L. Casey. Depts. of Neurology & Physiology, Univ. of Michigan and Neurology Research Laboratories, VA Medical Center, Ann Arbor, Mi 48105.

We have shown (Lin et al, this meeting) that in the rat, cocaine-HCl (25 mg/kg I.P.) is a rapid onset (< 10 min.), centrally acting analgesic that is neither sedative nor opiate-mediated. We are studying the possible neurophysiological basis for this effect through extracellular recording of changes in the spontaneous and evoked activity of antidromically identified bulboreticular neurons in rats anesthetized with chloral hydrate.

Twenty-three neurons were recorded from the medial medullary raphe, gigantocellular, or paragigantocellular nuclei. Of 20 spinally projecting neurons, 8 showed a prolonged increase, 2 a decrease, and 10 no change in the level of spontaneous activity within 10 minutes of cocaine injection. Thirteen neurons were responsive to noxious somatic stimulation. Of these, 70% (9) reduced their responsiveness after cocaine, with the remainder showing an increase (2) or no change (2). The increased responsiveness of one of these neurons was abolished within 3 minutes of the administration of chlorpromazine (3 mg/kg I.P.). The three remaining bulboreticular neurons projected rostrally through the medial forebrain bundle and showed immediate (<5 min.) cocaine-induced increases in spontaneous activity with a concurrent reduction in responsiveness to noxious stimulation.

These findings provide direct evidence that cocaine, in doses analgesic for the rat, affects both spontaneous and nociceptively-evoked activity of caudally and rostrally projecting bulboreticular neurons over a time course paralleling the behavioral effects. The observation that somatic responsiveness was reduced while spontaneous activity was unchanged or increased in most cells suggests a remote or presynaptic inhibition.

Supported by NIH, NSF and Veterans Administration.

440.3 MUTUAL POTENTIATION OF ANTINOCICEPTIVE EFFECTS OF COCAINE AND MORPHINE . S. Lei* and G. L. Wilcox, Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

It is well known that either noradrenergic or opioid agonists alone inhibit spinal nociceptive transmission to ascending systems. Further studies in this and other laboratories have indicated that these two classes of spinally active antinociceptive agents not only posess independent antinociceptive effects, but also show mutual potentiation. Most studies of opioid-noradrenergic synergism have involved morphine together with norepinephrine or clonidine. The present study sought to examine the interaction between morphine and cocaine, the latter agent having abuse liability, indirect noradrenergic agonist effects and local anesthetic effects. Naloxone and phentolamine were used to identify the receptor populations involved, and substance P (SP) was injected intrathecally (i.t.) to elicit scratching behavior.

Male mice (unanesthetized, 17-23 g) were given intrathecal (i.t.) injections by percutaneous lumbar puncture. This procedure involves directing a 30-gauge needle into an intervertebral space at approximately the level of the 5th or 6th lumbar verebrae. Intrathecal application of SP in the mouse results in a caudally directed biting and scratching behavior possibly indicating nociception.

SP-elicited behavior was inhibited significantly by cocaine in a dose dependent manner in a dose range from 1 to 5 μg. The inhibitory effect of cocaine (4μg, i.t.) on SP-elicited behavior was antagonized by co-administration of 0.1μg phentolamine.

Morphine (0.1μg) shifts the cocaine dose response curve about 10 fold to the left. Cocaine (0.5μg and 2.5μg) shifted the morphine dose response curve about 5 fold and 10 fold, respectively, to the left. The combined effect of morphine (0.5 μg, i.t.) and cocaine (0.5 μg, i.t.) was not blocked by 1 mg/kg naloxone (s.c.) but was significantly blocked by 1 μg phentolamine (i.t.).

Subcutaneous injection of morphine (2 mg/kg, a dose having no significant effect on SP-elicited behavior) increased the effect of cocaine (2.5 μg, i.t.), but complete dose response curves have not been completed. The combined effect was reduced by subcutaneous injection of naloxone (1 mg/kg).

These results suggest that cocaine exerts spinal antinociceptive effects though α-adrenergic receptors and, similar to direct-acting noradrenergic agonists, potentiates the spinal antinociceptive effects of morphine.

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440.4 MORPHINE INCREASES SUBSTANCE P IN THE DORSAL HORN DURING A NOCICEPTIVE STIMULUS. K.E. McCarson, B.D. Goldstein, M.L. Kirby. Departments of Pharmacology and Toxicology and Anatomy, Medical College of Georgia, Augusta, GA 30912.

The undecapeptide Substance P (SP) found in primary afferent terminals has been proposed to be a mediator of nociception in the dorsal horn of the spinal cord. Formalin injected into the hindpaw of a rat as a chemical nociceptive stimulus has been shown to increase the levels of SP as determined through quantitative immunohistochemistry. Morphine exerts an antinociceptive effect which may be due to its ability to bind to opiate receptors found on primary afferent terminals. In order to study the effect of morphine on SP levels in the dorsal horn, female Wistar rats were pretreated with subcutaneous injections of morphine sulfate (MS) 10mg/kg or saline 1.0 ml/kg. Rats were anesthetized with urethane and 5% formalin or saline (0.4 ml) was injected into the plantar aspect of the right hindpaw. Perfusion with 4% paraformaldehyde and removal of the lumbar enlargement of the spinal cord followed after 1 hour. SP was quantitated using immunohistochemical staining and manual photometry. Animals were grouped according to the pretreatment and the substance injected into the hindpaw. The four groups were saline-saline (pretreatment-hindpaw), MS-saline, saline-formalin, and MS-formalin. The results show that in MS-formalin animals SP increased by 53% in the ipsilateral dorsal horn compared to saline-saline animals. This increase was somewhat less than the sum of those increases found in saline-formalin animals (34%) and MS-saline animals (36%) as compared to saline-saline animals. In summary, these results confirm that SP in the dorsal horn can be increased by a chemical nociceptive stimulus and show that pretreatment with morphine produces an acute increase in SP in the dorsal horn. These data also show that morphine can further increase SP in the dorsal horn during a nociceptive stimulus. Supported by NS18664.

440.5 SYSTEMIC ADMINISTRATION OF NALOXONE OR NALTREXONE PRODUCES ANALGESIA IN BALB/c MICE IN THE FORMALIN PAIN TEST. A.L. Vaccarino, R. Melzack* and R.A.R. Tasker*. Department of Psychology, McGill University, Montreal, Quebec, H3A 1B1.

Systemically-administered naloxone usually produces hyperalgesia or no change in nociceptive responding depending on the animal species used and/or the pain test employed. We have recently found, however, that two opioid antagonists, naloxone and naltrexone, both produce a dose-dependent analgesia in the formalin pain test using an inbred strain of albino mouse.

In the first experiment, Female Balb/c, C57/BL and CD1 mice (18-22 grams) (N=30 for each strain) were injected s.c. with naloxone HCl in saline (0.1-10.0 mg/kg) or saline alone, and tested for analgesia 15 minutes later using the formalin pain test. Formalin testing involved injecting 20 μl of 5% buffered formalin acetate s.c. into the plantar surface of the animal's hind paw, and continuously rating the animal's pain behaviour (time spent licking the injected paw) for the subsequent 60 minutes. All mice were extensively habituated prior to testing. Naloxone produced a statistically significant dose-dependent analgesia in the Balb/c mice, with an ED50 of 0.24 mg/kg and almost total analgesia at doses of 1 mg/kg or greater. No changes in pain behaviour were observed in the C57/BL or CD1 strains of mice. We believe this to be the first report of analgesia following administration of doses of naloxone normally used for opioid antagonism. To determine if this effect was specific to the formalin test, the three strains of mice (N=18 for each strain) were injected s.c. with naloxone HCl and tested 15 minutes later in the tail-flick test (water at 49° C). Naloxone had no analgesic action in this test in any of the strains; however, hyperalgesia was observed in the CD1 strain. This latter finding is consistent with other reports. In a final experiment, the analgesic action of naltrexone was investigated in the Balb/c strain (N=30) using the formalin test. The procedure was the same as in the first experiment. Naltrexone showed a clear and statistically significant analgesia, with an ED50 of 0.05 mg/kg and almost total analgesia at doses of 0.1 mg/kg or greater. These data show clearly that administration of normal doses of opioid antagonists produces analgesia in the formalin test using Balb/c mice. Experiments designed to investigate the mechanisms underlying this action are currently underway. Supported by NSERC grant A7891.

440.6 PERTUSSIS TOXIN INHIBITS ANTINOCICEPTION PRODUCED BY INTRATHECAL INJECTION OF MORPHINE, NORADRENALINE AND BACLOFEN. J. Sawynok, K. Hoehn*, and A. Reid* (SPON: M.G. Murphy), Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada.

Activation of opiate and α-adrenergic receptors inhibits adenylate cyclase in cultured neuroblastoma-glioma hybrid cells, and this inhibition is blocked by pertussis toxin (J. Biol. Chem. 1983, 258:4870). Baclofen-induced inhibition of adenylate cyclase in cultured granule cells also is blocked by pertussis toxin (J. Pharmacol. Exp. Ther. 1986, 239:568). In spinal cord ganglion cultures, pertussis toxin blocks depressant effects of morphine and noradrenaline on dorsal horn network responses (Brain Res. 1987, 400:185), while in cultured dorsal root ganglion cells, pertussis toxin blocks noradrenaline and $GABA_B$-receptor inhibition of calcium channels (Nature 1986, 319:670). Such observations suggest involvement of the inhibitory guanine nucleotide regulatory unit and possibly adenylate cyclase in spinal actions of morphine, noradrenaline and baclofen. In the present study, we examined the effect of intrathecal (i.t.) pretreatment with pertussis toxin on antinociception produced by i.t. injection of morphine, noradrenaline and baclofen. Rats were implanted with chronic indwelling i.t. catheters and 5-7 days later pertussis toxin (0.25-0.75 μg) or vehicle (phosphate buffer) injected intrathecally. Antinociception was tested 2-7 days later using the tail flick test. After initial baseline latencies were determined, test drugs were injected and tail flick latencies measured at 15 min intervals for 60 min. Data was converted to an Antinociceptive Index (AI) score (area under curve) and in the table is expressed as % control responses in vehicle-pretreated rats tested on the same day (n in brackets).

Test agent	day 2	day 4 or 5	day 7	PT dose
Morphine 3-7.5 μg	46 ± 15(15)	32 ± 13(15)	35 + 9(14)	0.5 μg
AI 8.1-9.2 sec	< .05	< .05	< .02	
Noradrenaline 20 μg	14 ± 5(7)	10 ± 4(7)	21 ± 11(7)	0.5 μg
AI 15.2-18.1 sec	< .01	< .001	< .001	
Baclofen 0.3-0.6 μg	47 ± 17(11)	30 ± 10(11)	48 ± 13(11)	0.25 μg
AI 7.7-14.8	< .05	< .001	NS	
	75 ± 20(12)	53 ± 16(9)	38 ± 15(7)	0.75 μg
	NS	< .05	< .01	

The table indicates pertussis toxin inhibited antinociception produced by all three agents. These results suggest involvement of the inhibitory guanine nucleotide regulatory unit in the spinal antinociceptive action of morphine, noradrenaline and baclofen. However, the involvement of adenylate cyclase remains to be determined. (Supported by MRC Canada)

440.7 **THE EFFECT OF MORPHINE ON PERIAQUEDUCTAL GRAY NEURONS MAINTAINED IN VITRO.** **S.A. Chandler* and M.M. Behbehani (SPON: D.L. Ross). Department of Physiology and Biophysics, U. Cincinnati College of Medicine. Cincinnati, OH 45267-0576.**

In the past three decades it has been established that injection of morphine (MO) into the PAG produces strong and long lasting analgesia. Although the specificity of the action of morphine has been well established, the mechanism by which it produces analgesia is not clear. Experiments using electrical stimulation and injection of excitatory neurotransmitters such as glutamic acid and substance P into the PAG suggest that excitation of PAG neurons can consistently lead to analgesia whereas lesion of PAG or injection of local anesthetic into this region seldom leads to modulation of pain perception. Since in most CNS sites, injection of MO produces a reduction in the firing rate and excitability of the neurons, it has been suggested that MO injected into the PAG produces analgesia through the process of disinhibition. To test this hypothesis, we have examined the effect of morphine on PAG slices maintained in normal physiological saline solution (PSS) and in calcium free solution that instead of calcium contained cobalt (PSS-Co).

Adult male Sprague-Dawley rats were used. Animals were anesthetized with ether and then decapitated. The brain was removed and a three mm section surrounding the sagittal sinus was cut. This tissue was then sectioned into 450 micron slices. After incubation for one hour, one slice was placed in the recording chamber and was covered by a nylon mesh. The tissue was continuously perfused with oxygenated PSS at a rate of 3 ml/ min. Extracellular recording was made from all regions of PAG using a glass electrode. Morphine was applied directly to the cell or through bath application. The specificity of the MO effect was tested by pretreatment with naloxone.

The effect of MO was examined in 233 cells in 85 animals. Only 40 (17%) of the cells were responsive to morphine. In this population 9 cells were excited by morphine and the rest were inhibited. The inhibitory effect of morphine was dose dependent and could be reversed by naloxone. The excitatory effect of morphine was seen at a higher dose and was only partially reversed by naloxone. Both the inhibitory and the excitatory effects of morphine were observed when the tissue was incubated in PSS-Co solution.

It is concluded that the major effect of morphine on the PAG neuron is inhibition. The observation that the MO effect could be detected when all synaptic transmission had been blocked, indicate that MO produces its action through a post synaptic mechanism. These studies suggest that the analgesic effect of morphine is likely to be due to inhibition of inhibitory neurons that project outside the PAG rather than disinhibition of PAG interneurons. Supported by PHS Grant NS 20643.

440.8 NOCICEPTIVE ASSESSMENT DECREASES THE SYNERGISTIC EFFECT OF CONCURRENT SPINAL AND SUPRASPINAL MORPHINE ADMINISTRATION. J. Siuciak* and C. Advokat (SPON: D. Levy) Dept. of Pharmacology, U. of I. College of Medicine, Chicago, Il. 60612

Recent studies have characterized the complex relationship between the spinal and supraspinal processes underlying opiate analgesia and tolerance. These studies have demonstrated that concurrent morphine injections onto the spinal cord and in the cerebral ventricles produces a multiplicative analgesic effect (Yeung and Rudy, 1980). Fujimoto and associates (Roerig et al, 1984) further demonstrated that tolerance, induced by morphine pellet implantion in mice, reduced this multiplicative interaction to an additive relationship. The present studies were designed to extend this analysis by evaluating the influence of both behavioral and pharmacological pretreatment on this multiplicative phenomenon, and to determine whether the effect would be obtained from another supraspinal site, the periaqueductal gray (PAG).

Rats received concurrent injections of morphine into the intrathecal space (IT) and the PAG. The antinociceptive effect of these dual injections was assessed with the tail flick withdrawal test. Dose response curves were constructed by administering different combinations of doses into the PAG and intrathecal space (PAG:IT ratios of 1:0, 1:1, and 0:1). Statistical analysis of these curves confirmed the multiplicative phenomenon. That is, the dose response curves produced by the 1:1 (PAG:IT) ratio was shifted significantly to the left of the line produced by the 1:0 ratio.

Additional experiments investigated the effect of tolerance to systemic morphine on this synergism. Rats that had received a dual morphine injection at the 1:1 dose ratio were injected daily with either morphine (3 mg/kg, sc) or saline, and tested on the tail flick for six consecutive sessions, i.e. until the morphine treated rats became tolerant. At that time they received a second concurrent morphine injection at the same dose ratio (PAG:IT = 1:1). In response to this challenge there was a significant decrease in the synergistic effect in both morphine and saline pretreated groups. This reduction was significantly greater in the morphine than the saline pretreated animals.

These results demonstrate that the multiplicative interaction can be obtained from the PAG and that both behavioral as well as pharmacological variables contribute to the decrease induced by tolerance to systemic morphine. Supported by NIDA grant DA02845.

440.9 THE EFFECTS OF MU- AND KAPPA-OPIOID RECEPTOR AGONISTS ON SCIATIC NERVE STIMULATION-EVOKED POTENTIALS IN THE RAT. K. W. Locke*, J. A. Little* and S. Fielding (SPON: C. P. Smith). Department of Biological Research, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

The potentials elicited by stimulation of the isolated sciatic nerve in the hindlimb of the anesthetized rat were recorded from electrodes placed over the surface of the somatosensory cortex. Recordings were made bilaterally and the response to the stimulation was observed contralateral to the stimulation site. The potentials evoked by sciatic nerve stimulation were insensitive to neuromuscular blockade by d-tubocurarine (0.8 mg/kg sc), suggesting that the potentials were sensory in nature. Typical averaged recordings revealed at least two major negative potentials, a small early potential (latency = 11-13 ms) and a larger later potential (latency = 17-19 ms).

The effects of a number of opioids on the evoked potentials were compared. In general, analgesic doses of mu-opioid agonists (i.e., morphine 3 mg/kg sc and levorphanol 1 mg/kg sc) reduced the amplitude of the early negative potential, enhanced the amplitude and prolonged the latency of the larger later potential and often unmasked positive and negative potentials with latencies greater than 25 ms. The mixed mu- and kappa-opioid agonists, pentazocine (30 mg/kg sc) and butorphanol (2 mg/kg sc) reduced the amplitude of the early potential and enhanced the amplitude and prolonged the latency of the later potential but also broadened the later potential (by enhancing the amplitude of a second later potential). In contrast, relatively "selective" kappa-opioid agonists produced a wide variety of changes in the evoked potentials. Ethylketocyclazocine (0.3-1 mg/kg sc) produced effects similar to those produced by morphine. Ketocyclazocine (10 mg/kg sc) depressed the amplitude of the later negative potential and cyclazocine (3-10 mg/kg sc) and U50,488H (3-10 mg/kg sc) produced no significant effects on the evoked potentials. The changes induced by the mu-opioid agonists were readily reversed by naloxone (0.3 mg/kg sc) whereas the effects of the opioids with significant affinity for the kappa receptor were not. The reasons for the differences between the kappa-opioid agonists are unclear. However, the measurement of sciatic nerve stimulation-evoked potentials appears to be an effective method of differentiating among classes of opioid agonists in vivo.

440.10 MORPHINE-6-GLUCURONIDE: ANALGESIC EFFECTS AND RECEPTOR BINDING PROFILE IN RATS. F.V. Abbott[1] and R.M. Palmour[2]. Dept. of Psychiatry [1,2], School of Nursing[1] and Centre for Human Genetics[2], McGill Univ. Montreal, PQ, Canada H2A 2A7.

Morphine-6-glucuronide (M-6-G) is a minor metabolite of morphine but serum levels of M6-G are higher than those of morphine in cancer patients (Sawe, 1986). While its role in analgesia in man is not known, M-6-G is a potent analgesic in mice (Shimomura et al, 1971). The present experiments investigated the analgesic activity of M-6-G in heat and tissue injury-induced pain in rats and its binding characteristics in rat brain membranes.

Analgesia assays: M-6-G was injected intraventricularly (icv) via chronically implanted cannuli or s.c. In heat pain (tail immersion test), both routes produced peak effects at 40-60 min. The dose-effect relationship in heat pain consisted of 2 components; in the lower portion analgesia plateaued after the tail withdrawal latency was increased by 2-3 fold at doses of 52 pM icv or 4.2 uM/kg sc. Doses greater than 416 pM icv or 8.3 uM/kg produced further increases in the tail withdrawal latency and also marked catalepsy and respiratory depression. Naloxone (0.1 mg/kg sc) shifted the lower portion of the dose-effect relation by a factor of 22. Because of the toxicity of M-6-G, it was not possible to determine the naloxone induced shift for the upper portion. M-6-G icv was 72 (lower portion) to 288 (upper portion) times as potent as morphine given by the same route.

In tissue injury-induced pain (formalin test) M-6-G produced a steep simple dose-effect relation with an ED_{50} of 208 pM icv and 12.4 uM/kg sc. Naloxone (0.1 mg/kg sc) shifted the dose effect relation for icv M-6-G by a factor of 5. M-6-G icv was 79 times as potent as icv morphine.

In vitro receptor binding: M-6-G has biphasic effects on the binding of opiate agonists and antagonists in rat brain membranes (rapid filtration assay). Over the dose range 10^{-9} -10^{12} M, M-6-G potentiates the binding of ^{3}H-etorphine, ^{3}H-Naloxone and ^{3}H-dihydromorphine (maximum 158%, 135%, 150%, respectively). Between 10^{-5} and 10^{-8}M, M-6-G dose-dependently displaces binding of these ligands, with IC_{50} values ranging between $2x10^{-6}$ and $5x10^{7}$. If the nominal EC_{50} for binding of morphine to its receptor is about 10^{8}M, then, on the basis of the behavioral potencies reported here, M-6-G must exert physiological effects between 10^{-9} and 10^{-11} M.

M-6-G is a very potent analgesic when administered centrally. Despite its polar nature, it is equipotent with morphine by the sc route. The naloxone antagonism is similar to that seen 60 min after morphine administration but less than that observed at 30 min. when brain morphine levels are higher (Abbott et al, 1986). This suggests that the morphine time-effect relation in rats reflects 2 processes involving different receptor interactions.

440.11 INTRACEREBROVENTRICULAR MORPHINE RESTORES THE BASIC SOMESTHETIC ACTIVITY OF DORSAL HORN CONVERGENT NEURONS IN THE RAT.
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It is well established that, in rodents, intracerebroventricular (ICV) administration of morphine and related compounds results in powerful analgesia. Recently ICV morphine has been introduced as a form of pain therapy in humans, especially for the relief of intractable cancer pain. However, the neurophysiological basis of such an action remains to be elucidated. Convergent neurons are one of the prime candidates for processing nociceptive information at both spinal and trigeminal levels. The convergence of various types of information from both noxious and innocuous origins onto these neurons predisposes this neuronal population to a global reception of sensory information which could be interpreted by the higher centers as a basic somesthetic activity (BSA).

We have proposed that the occurence of a nociceptive event on an area of the body would perturb this BSA in two complementary ways: by activation of the corresponding segmental pool of neurons and by an inhibition mediated by Diffuse Noxious Inhibitory controls (DNIC) which involve supraspinal structures of the remaining extrasegmental neuronal population. The contrast message between the smaller excited and the large inhibited pool of neurons may well constitute a nociceptive signal information to the brain.

We present here evidence that morphine (10 µg) administered within the third ventricle in anesthetized rats completely blocks DNIC acting on the BSA. DNIC were triggered by the immersion of the tail in a 48°C waterbath, and BSA was simulated by responses of convergent neurons recorded at the lumbar level to innocuous stimuli produced by means of a motor-driven paint-brush applied regularly (2-3 Hz) on the excitatory receptive field located on the ipsilateral hindpaw. This action of morphine was observed within 10 min following the micro-injection,was long-lasting (i.e. > 60 min) and was antagonized by systemic naloxone (0.4 mg/kg). It is then proposed that analgesia induced by ICV morphine could results, at least in part, from a restoration of the BSA. In addition, autoradiographic controls using ^{3}H-morphine showed that the diffusion of morphine was restricted to the hypothalamus around the tip of the canula, the periagueductal gray (PAG) and the floor of the fourth ventricle, three regions which have been implicated in morphine analgesia. No labelling was found over the lumbar spinal cord.

440.12 INTERACTIONS OF INTRATHECAL ANTI-SUBSTANCE P, MORPHINE AND NORADRENALINE ON MECHANICAL AND THERMAL ANALGESIA IN THE RAT.
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We have found that intrathecal (i.t.) administration of a rabbit antibody to substance P (AntiSP) produces analgesia to noxious mechanical stimulation but not to noxious thermal stimulation in the rat (In: Substance P & Neurokinins, 1987). Since i.t. morphine sulfate (MS) and noradrenaline (NA) inhibit release of SP in spinal cord (Brain Res. 376:268, 1986), we tested the possible interaction of i.t. AntiSP with MS and NA on the response to noxious mechanical (paw pressure) or noxious heat (tail flick) stimulation. I.t. cannulas were implanted into 24 male rats which were allowed one week to recover from surgery. The experimental design was a within-subjects Latin Square and every animal received every treatment with a 2-4 day interval between all tests. Six treatment conditions were tested and rabbit AntiSP (Immuno Nuclear) or normal serum (10µl of a 1/10 dilution) were always injected first. Rats were then immediately injected with either NA (10µg in 10µl), MS (1µg in 10µl) or saline (10µl) and the cannula flushed with 7µl of saline. The two modalities (heat and pressure) were tested in tandem respectively and two pre-injection determinations were made prior to the i.t. injections. Five post-injection tests were conducted at 7.5, 15, 22.5, 30, and 45 minutes. The data were analyzed by a 2 x 3 ANOVA with repeated measures on all factors. Low fixed doses were used because the expected direction of interaction was an increase in analgesia. At these doses, the analgesic effects of AntiSP, NA and MS were comparable in the pressure test, while in the tail flick test, the effects were NA > MS > AntiSP. Relative to control serum, a modality-specific effect of AntiSP was seen in that the rats showed a significant analgesia to pressure, but AntiSP had only a modest effect on the tail flick response in that the downward drift of control latencies was attenuated. When NA was combined with AntiSP injections, the effects on the response to pressure and temperature were increased at 22.5 minutes post-injection. In contrast, the effects of MS plus AntiSP on the response to pressure were less than additive for all post-injection intervals, while for the tail flick test, the effects were additive. These data suggest that NA and MS interact with SP in the spinal cord by different mechanisms. Also, both MS and AntiSP have a much greater effect on noxious mechanical rather than thermal stimulation; whereas, NA is equally effective for both modalities. These results with AntiSP support the concept of a modality specific role for SP. (Supported by MRC of Canada and Dalhousie Medical Research Foundation).

440.13 ANTINOCICEPTION PRODUCED BY SYSTEMIC MORPHINE IS BLOCKED BY INTRANIGRAL INJECTION OF NALOXONE.
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Bilateral microinjection of morphine into the substantia nigra (pars reticulata) of rats produces antinociceptive effects on the hot-plate and tail-flick tests (Baumeister et al., Brain Res., in press). This finding suggests that nigral opiate receptors may mediate the antinociceptive effects produced by systemic morphine. The following study was designed to test this hypothesis. Bilateral guide tubes (22 gauge) were implanted 1mm above the substantia nigra (A 2.7mm, L±2.1mm, V 8mm) of male Sprague Dawley rats (250-350g) under Ketamine (150mg/kg, im) anesthesia. One week later these animals were injected subcutaneously with either morphine sulfate (5mg/kg) or saline. Twenty minutes after the subcutaneous injection animals received bilateral intranigral injections of either saline (0.5µl) or naloxone HCl (10µg in 0.5µl saline). Ten minutes later animals were tested on the tail-flick and hot-plate tests as previously described (Baumeister et al., Brain Res., in press). The mean paw-lick and tail-flick latencies (in seconds) for each of the four groups are shown in the table below. As can be seen, systemic morphine had a strong antinociceptive effect on both tests. These effects of morphine were completely blocked by intranigral injection of naloxone. Naloxone alone did not alter responses on either test. These results suggest that the antinociceptive effects of systemic morphine are mediated by opiate receptors in the substantia nigra. Supported by US Public Health Service grant HD-21560.

Group			
Systemic Injection	Intranigral Injection	Paw-lick Latency	Tail-flick Latency
Saline	Saline	7.1±1.3	3.2±0.1
Saline	Naloxone	12.9±4.4	2.8±0.2
Morphine	Naloxone	9.2±1.4	2.4±0.2
Morphine	Saline	30.0±0.0*	10.0±0.0*

*p<.05 compared to all other groups. There were 5 animals in each group.

440.14 TOLERANCE TO OPIATE ANTINOCICEPTION IN SPINALLY TRANSECTED RATS.
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These studies represent part of a series of investigations designed to examine the functional role of the spinal cord in tolerance to opiate antinociception. The nociceptive response was defined as the latency of tail withdrawal (tailflick, TF) in rats, elicited by a high intensity thermal stimulus. In the first study the effect of a spinal transection on opiate antinociception in morphine tolerant rats was examined. Tolerance was produced in one group of rats by repeated morphine injections (3.0 mg/kg sc) and TF tests. A second group received an equal amount of saline pretreatment and tests. Once tolerance developed, one half of the rats in the morphine and saline pretreated groups were spinalized (T6-T9) under ether anesthesia; the other half were left intact. On the following day all animals received a final morphine injection and test.

The results indicated that morphine pretreated rats remained tolerant relative to nonpretreated rats for at least one day after a spinal transection. However, the opiate response of saline pretreated rats was also significantly reduced after spinalization relative to that of nonpretreated spinal rats. These data suggested that prior behavioral assessment even without prior drug exposure, reduced the sensitivity of the spinal cord to morphine, but that this decrease was only expressed after a spinal transection.

To test this conclusion the experiment was repeated in rats who were spinally transected before they received morphine or saline. Morphine administration produced an anti-nociceptive response in spinal rats which declined to tolerant levels during successive test sessions. Both spinal and intact rats were capable of retaining this tolerant response for at least one week after the last injection. However, saline pretreatment did not reduce the response of spinal rats to a subsequent morphine challenge. These data indicate that the spinal cord is capable of developing pharmacological tolerance to opiates, but that behavioral influences on tolerance are mediated by supraspinal structures. Supported by PHS grant DA-02845.

440.15 MORPHINE'S SITE(S) OF ACTION IN THE NEUROCIRCUITRY FOR THE NOCICEPTIVE JAW-OPENING REFLEX: COMPARISON TO CLONIDINE. A.L. Curtis and J. Marwah. Neuroscience Laboratory, Dept. of Pathol., Univ. Med. & Dent. of New Jersey, Camden, N.J. 08103.

The rat oral-facial response to maxillary tooth pulp stimulation (TPS) is the jaw-opening reflex(JOR) which was utilized in the present study as a nociceptive paradigm. The digastricus (a jaw opening muscle) electromyogram (dEMG) threshold (dEMGT) and amplitude (dEMGA) were utilized as indices of nociception. Simultaneous recording of the mean arterial pressure (MAP) was also performed. Both clonidine and morphine dose dependently and significantly elevated the TPS-dEMG threshold and depressed the TPS-dEMG amplitude. Sustained depressor conditions were induced by both agonists; however, a transient pressor response was elicited by clonidine, while a transient depressor response was elicited by morphine. Yohimbine (an alpha$_2$ adrenoceptor antagonist) but not prazosin (an alpha$_1$ adrenoceptor antagonist) or naloxone (a mu receptor antagonist) antagonized the antinociceptive effects of clonidine. Conversely, naloxone but not yohimbine antagonized the antinociceptive effects of morphine. However, yohimbine did antagonize the depressor effect of morphine, which was further antagonized by naloxone. The antinociceptive actions of morphine and clonidine did not include direct effects on the digastric muscle or the neuromuscular junction. It is concluded that morphine's antinociceptive effects are mediated by central mu receptors that do not appear to involve alpha$_2$ adrenoceptors. (Supported in part by grants from NIDA (DA - 04158).

440.16 **LOW FREQUENCY ELECTRO-ACUPUNCTURE ANALGESIA IN RATS: EVIDENCE FOR ENDOGENOUS OPIOIDS INHIBITING EFFECTS**
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Low frequency electro-acupuncture (EA) analgesia in rats is thought to be mediated by endogenous opioids because naloxone antagonized 2 Hz EA analgesia and partially reversed 2-15 Hz EA analgesia (1 and 20 mg/kg respectively, Han et al. Acta Physiol. Sinica 38(5):475 1986). We conducted experiments with members of Han's laboratory in the U.S. and in their laboratory in China using the exact methods of Han et al. (measurement of tail flick latencies at the end of each 10 min period of 1, 2, or 3 volts EA stimulation of the hind limbs, and every 3 min for 12 min thereafter). In no experiment was opiate antagonist reversal or reduction of EA observed; More specifically, we found the following:

1. Naloxone (10 and 30 mg/kg) and naltrexone (2 and 10 mg/kg) potentiated 2 Hz and 2-15 Hz EA analgesia in most experiments.
2. The potentiation occurred independently of the geographic location of the experiment (China or America), the strain of rat (Chinese or American), tail temperature, age, or weight. In some instances the females were unaffected by the opiate antagonist pretreatment while the males showed a potentiation.
3. Spinalization or DLF lesions abolished all analgesia indicating that supraspinal structures are involved in mediating the analgesic effect.
4. Hypophysectomy or adrenalectomy of male rats increased the level of EA-induced analgesia independently of opiate antagonist pretreatment suggesting that (a) the observed analgesia is not dependent on the pituitary-adrenal axis for expression and (b) a hormonal factor may be released from one of these glands (possibly opioids from the pituitary and adrenal) which would inhibit EA-induced analgesia.

Taken together these results suggest: (1) The phenomenon is highly variable from one experiment to the other, even with rigorous replication of the methods. An explanation for this variability may be that EA activates multiple parallel and conflicting neural circuits which interact and ultimately modulate the analgesic outcome. This multiple activation could mask an opioid-dependent analgesic system which therefore cannot always be detected. Other possible variables which have not been studied here include the effects of seasonal and circadian fluctuations. (2) EA activates an opiate system which inhibits the development of analgesia. (3) This model of EA analgesia is probably not an appropriate model of human EA because the high intensity of the stimulation produces perceptible discomfort of the animals and because low frequency EA analgesia in humans is generally blocked by naloxone.
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440.17 CONTRASTING EFFECTS OF ACUTE VS. CHRONIC PROGLUMIDE TREATMENT UPON SPINAL MORPHINE ANTINOCICEPTION.
D.E. Kellstein and D.J. Mayer, Department of Physiology, Medical College of Virginia, Richmond, VA 23298.

Considerable evidence suggests that cholecystokinin octapeptide (CCK-8), a putative CNS neuromodulator, functions as an endogenous antagonist of opioid analgesia. Previous studies in this laboratory demonstrated that acute administration of proglumide (PGM), a CCK-8 receptor antagonist, enhances both intrathecal (IT) and periaqueductal gray (PAG) morphine analgesia (Watkins et al, Brain Res. 327, 1985), suggesting that PGM may be a useful adjunct to opioid analgesics in the management of chronic pain. The present study investigated the interaction of chronic PGM with IT morphine antinociception.

Under pentobarbital anesthesia, rats were implanted with IT catheters which terminated at the lumbar enlargement. Following a one-week recovery period, morphine (1.0 µg, IT) analgesia was evaluated weekly with a tail-flick assay during, and after, 22 daily injections of PGM (0.02 µg, IT). On day one, PGM pretreatment reliably ($P<0.01$, area under the curve) enhanced morphine antinociception compared to saline pretreatment, but this facilitation was absent on days eight and 15 of PGM treatment. On day 22, however, PGM reliably ($P<0.01$) attenuated IT morphine. Following termination of daily PGM injections, morphine analgesia was unaltered by PGM pretreatment on days 29 and 36.

Weekly PGM treatment exhibited similar effects. Morphine analgesia was initially (i.e., day one) enhanced ($P<0.01$) by PGM pretreatment, but this facilitation was lost by day eight and remained absent for the duration of the study (i.e., up to day 36). However, inhibition of morphine antinociception was not observed with this treatment paradigm.

The present results demonstrate that whereas acute IT PGM administration enhances IT morphine, chronic treatment causes loss of facilitation and eventual attenuation of IT morphine analgesia. These findings suggest that CCK-8 blockade with PGM (or other CCK-8 antagonists) may not be an effective adjunct to opioids in the treatment of clinical chronic pain and may be deleterious. Although the mechanism(s) of this interaction have not been elucidated, tonically active spinal antinociceptive pathways are probably not affected, since nociceptive thresholds were unaltered in animals receiving chronic IT PGM alone. Studies are in progress to determine if a similar interaction occurs between chronic PGM and morphine in the PAG.

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440.18 SPINAL ANTINOCICEPTIVE PROPERTIES OF MORPHINE, KETAMINE, SEROTONIN AND NOREPINEPHRINE. T. Crisp, J.M. Perrotti* and D.J. Smith. Dept of Anesth, WVU Med Ctr, Morgantown, WV 26506

Morphine (MS), Ketamine (KH; a phencyclidine analog), serotonin (5-HT) and norepinephrine (NE) have all been shown to produce analgesia in rats following intrathecal (i.t.) administration (Abstr. Soc. Neurosci. 12:619, 1986; JPET, 208:446, 1979). Additionally, analgesia produced by MS and KH appears to incorporate a local serotonergic component at the spinal level (Pain, 12: 57, 1982). The present study was designed to characterize opiate and monoamine dependency of the spinal analgesic actions of MS and KH.

Male Sprague-Dawley rats (325 ± 25 g) were cannulated with indwelling PE-10 catheters for i.t. injections. Nociception was assessed using the tail-flick latency (TFL) test, and dose-response curves were generated. Transient behavioral signs were also observed following the highest doses of KH, 5-HT and NE (e.g., tail rigidity, tremors, vocalization and hindlimb paralysis) but these signs had a very short duration (5-10 m) and were clearly dissociated from the drug-induced analgesic properties. IC_{50} values are presented below for the ability of s.c. naloxone, methysergide and phentolamine to block the analgesic actions of MS, KH, 5-HT and NE.

Analgesic Agent	Naloxone IC_{50} (mg/kg)	Methysergide IC_{50} (mg/kg)	Phentolamine IC_{50} (mg/kg)
MS (10nmol)	0.02	4.80	***
KH (1µmol)	0.3	*	**
(3µmol)	3.2	0.01	0.58
5-HT(1µmol)	-----	0.01	***
NE (30nmol)	-----	----	0.3

*** - no effect at the highest dose tested (10 mg/kg s.c.)
** - significant inhibition only at 10 mg/kg s.c.
* - complete inhibition occurs between 0.1 and 0.3 mg/kg s.c.

These data verify the involvement of a local serotonergic mechanism in the spinal antinociceptive actions of MS and KH. However, MS analgesia was less readily inhibited by methysergide (cf. KH and 5-HT) and was not altered by phentolamine. Thus, opiate mechanisms appear to be most prominent and essential for MS spinal analgesia. These data also demonstrated that the monoaminergic dependence of KH analgesia increases with high doses of the drug. In fact, noradrenergic systems that appear to be relatively unimportant at 1 µmole (i.t.) of KH seem to be strongly recruited at 3 µmoles. Furthermore, it appears that an opiate receptor variant (e.g. PCP/sigma site) may also be involved in KH analgesia since higher doses of naloxone are required to reverse the effect of KH than are needed for MS.
Supported by WVU Med. Corp. and NIH grant GM30002.

440.19 POTENTIATION OF MORPHINE ANALGESIA BY D-AMPHETAMINE IS MEDIATED BY NOREPINEPHRINE AND NOT DOPAMINE. S.E. Izenwasser and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Morphine and other opioid analgesics will raise the threshold for escape from aversive electrical stimulation delivered to the mesencephalic reticular formation (MRF). This effect is markedly potentiated by administration of low doses of d-amphetamine, which alone have no effect on the escape threshold (Sasson, et al., Psychopharmacology 90:163-165, 1986). Because amphetamine does not act selectively on a single neurotransmitter system, it is difficult to draw conclusions about the mechanism by which this potentiation occurs. In order to study the roles of dopamine and norepinephrine in mediating the potentiation of morphine analgesia by d-amphetamine, the effects of both amfonelic acid, an indirect dopamine agonist, and nisoxetine (Lilly, Corp.), a selective norepinephrine reuptake blocker, were determined alone and in combination with morphine using the brain stimulation escape pain model.

Bipolar stainless steel electrodes were stereotaxically implanted into the MRF of male F344 rats who were then trained to turn a wheel manipulandum to escape from electrical stimulation to this brain site. Escape thresholds were determined using a modification of the psychophysical method of limits.

Amfonelic acid alone produced hyperalgesia, as evidenced by significantly lowered escape thresholds, and completely antagonized the threshold raising effect of morphine. Nisoxetine had no effect on the escape threshold when administered by itself; it, however, potentiated the analgesic effect of all doses of morphine when the two drugs were administered concomitantly. These findings suggest that the potentiation of morphine by d-amphetamine, which has been shown both in this pain model as well as in clinical studies (e.g., Forrest, et al., New Engl. J. Med. 296:712-715, 1977), is likely due to increased noradrenergic activity and not to activation of dopamine systems.

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NEURAL PLASTICITY IN ADULT ANIMALS III

441.1 PUP-INDUCED MATERNAL BEHAVIOR IN VIRGIN RATS IS ASSOCIATED WITH INCREASED FREQUENCY OF DYE COUPLING AMONG SUPRAOPTIC NUCLEUS (SON) NEURONS. B. K. Modney*, Q. Z. Yang and G. I. Hatton (SPON: J. I. Johnson). Dept. Of Psychology and Neuroscience Program, Michigan State University, East Lansing, MI 48824.

Ultrastructural changes have been shown to occur in the dendritic zone of the SON of both immediately postpartum and lactating, nursing mothers (Neurosci. 1984, 13, 769-779; see Taubitz et al. abstract). One salient feature of these conditions is the formation of dendritic bundles (direct dendritic membrane appositions) apparently due to the withdrawal of astrocytic processes which normally separate the dendrites from one another. Similar changes have also been found to occur in virgin rats induced by the presence of young pups to exhibit maternal behavior (Salm et al., Soc. Neurosci. Abstr. 1985, 11, 612).

We had also found that dye coupling, interneuronal transfer of Lucifer Yellow (LY), predominantly via dendritic interactions, was increased in lactating, maternally behaving rats (Hatton et al., Neurosci., in press). Thus it was of interest to investigate the possibility of a relationship between dye coupling and induced maternal behavior.

After 2 days of cage habituation, individually housed virgin rats (40-55 days old) were continuously exposed to 4 pups (2-11 days old). Daily 15 min behavioral observations were made of maternal behaviors. Animals met our maternal behavior criterion when they displayed all of the following for 3 consecutive days: nesting, pup retrieval, anogenital licking and crouching over the pups. A minimum of 9 days and a maximum of 22 days of pup exposure were required to meet criterion. 3-8 days of full maternal behavior were observed before animals were used in brain slice experiments. LY was injected intracellularly into SON neurons in horizontally cut hypothalamic slices from maternally behaving virgin females. Of 17 injections made to date, 11 were made in slices after 10 min of electrical stimulation (10 Hz) of the lateral olfactory trace (LOT) input to the SON. The remaining 6 cells were from unstimulated SONs. These 17 injections produced 13 single and 11 coupled dye filled cells. The dye coupling index (DCI; no. of dye coupled neurons ÷ total no. of dye filled neurons) for maternally behaving virgins was 0.53. Comparison with 74 injections (35 from stimulated and 39 from unstimulated (SONs) into SON neurons from non-maternal virgins (DCI=.24) yielded a significant difference (χ^2= 3.05, p<.01). A further difference not reflected in these numbers was that coupled cells from maternally behaving rats showed higher order coupling (triplets and quadruplets) which have been observed in lactating but not in virgin animals (see Yang & Hatton abstract).

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441.2 DYE COUPLING INCIDENCE AMONG SUPRAOPTIC NUCLEUS (SON) NEURONS IS INCREASED BY LATERAL OLFACTORY TRACT (LOT) STIMULATION IN SLICES FROM LACTATING, BUT NOT VIRGIN OR MALE RATS. Q. Z. Yang and G. I. Hatton. Neuroscience Program, Michigan State University, East Lansing, MI 48824.

Transfer of Lucifer Yellow (LY) from one neuron to another in the SON has been shown to occur with increased incidence among oxytocin neurons in lactating, nursing rats compared to virgins (Hatton et al., Neuroscience, in press). Since dye coupling is an indicator of electrical coupling, this supports the hypothesis that electrotonic conduction among SON neurons is part of the mechanism for synchronizing the burst of firing in oxytocin cells prior to the milk ejection reflex.

LOT stimulation has been shown to have predominantly excitatory effects on SON neurons, particularly oxytocin cells (Dougherty et al., Soc. Neurosci. Abstr. 1986, 12, 1256). This appears to be via mono- and di-synaptic connections from the main olfactory bulb to the SON dendritic zone (Hollowell et al., Soc. Neurosci. Abstr. 1986, 12, 1256). As part of our ongoing investigation of the SON, we noted that dye coupling tended to be more frequent in slices containing recorded neurons that were excited by LOT stimulation.

In the present study, LY was intracellularly injected into SON neurons of horizontally cut hypothalamic slices from 44 virgin female, 22 male and 27 lactating rats. Roughly half of the LY injections in each group were made without stimulating the LOT, while the other half were made after 10 min. of stimulation at 10 Hz. A dye coupling index (DCI) was computed for each condition. DCI= no. of dye coupled neurons ÷ total no. of dye filled neurons. For virgins, DCI= 0.21 (unstim.), 0.29 (stim.); males, DCI= 0.58 (unstim.), 0.58 (stim.); lactating, DCI= 0.43 (unstim.), 0.80 (stim.). The DCI was significantly increased by stimulation only in lactating rats, χ^2 = 9.33, p<0.005. DCIs for males and virgins differed at p<0.01. LOT stimulation in the lactating rat produced more dye coupling by increasing the higher order coupling, i.e. the number of neurons coupled to an injected cell, rather than by merely increasing the incidence of coupled pairs. In non-stimulated slices from lactating rats 21 LY injections resulted in 16 singles, 3 pairs and 2 triplets; whereas 27 injections in stimulated slices yielded 13 singles, 6 pairs, 4 triplets, 1 quadruplet, 2 sextuplets and 1 septuplet. The distribution of cells coupled to the injected neurons in virgins, but not in lactating rats was described by the Poisson distribution.

These results suggest that the electrical coupling among SON neurons can be rapidly modified through sensory input in lactating rats.

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441.3 ANTEROGRADE AND RETROGRADE TRACING STUDIES OF THE MAIN OLFACTORY BULB INPUTS TO RAT SUPRAOPTIC NEURONS. K.G. Smithson*, M.L. Weiss and G.I. Hatton (SPON: D. Tanaka). Neuroscience Program, Physiology Dept., College of Osteopathic Medicine, Michigan State Univ., E. Lansing, MI 48824-1117.

Earlier studies using either silver impregnation of degenerating terminals (J. Comp. Neurol. 1975, 161:31-56) or injections of free horseradish peroxidase (HRP; J. Comp. Neurol., 1978, 181:213-244) have indicated the existence of a main olfactory bulb (MOB) input to the region of the supraoptic nucleus (SON). More recent evidence (Hollowell et al., Soc. Neurosci. Abst., 1986, 12:1256), using the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHAL), has shown that the SON receives a direct input from the MOB. However, while PHAL labeling consistently revealed fibers and terminals around and within the nucleus, the labeling was relatively sparse. Previous electrophysiological evidence (Yang & Hatton, Soc. Neurosci. Abst., 1986, 12:1256) suggested that this connection should be robust (i.e., LOT stimulation activates many SON neurons). In an effort to address the disparity between data generated by these two approaches, we re-examined this connection.

Discrete pressure injections of 0.7% WGA-HRP (supplied by R.R. Miselis) were stereotaxically placed in the MOB to study the anterograde distribution of the tracer which was visualized with either TMB or DAB chromogens. These injections revealed thick fascicles coursing within the ipsilateral lateral olfactory tract. As these fascicles passed caudal to the piriform cortex they thinned appreciably while continuing to run along the ventral surface of the brain. Continuing caudally, a plexus of fibers projected dorsomedially to densely label the lateral margins of the posterior one-half of the SON. Fluoro-gold (1-2% in water, supplied by L. Schmued) or rhodamine-labeled microspheres were injected into the SON to study the afferent projection to this area. This resulted in retrograde labeling of mitral cells throughout the main olfactory bulb without apparent preferential distribution. The dense WGA-HRP labeling appears to distribute to the dendritic zone of the SON, confirming our observation from PHAL studies. Taken together with the magnitude of mitral cell labeling, the pattern of WGA-HRP labeling demonstrates a relatively large input into the SON. These findings are in better agreement with our earlier electrophysiological findings. Supported by NIH grants 09140 and 16942, NRSA Postdoctoral award NS 08125 to MLW and Medical Scientists Training Program fellowship to KGS.

441.4 DENDRITIC ZONE OF THE SUPRAOPTIC NUCLEUS (SON): ULTRASTRUCTURAL CHANGES IN MOTHERHOOD. I.L. Taubitz*, K.G. Smithson* & G.I. Hatton (SPON: G. Richmond). Neuroscience Program and Physiology Dept., Michigan State University, East Lansing, MI 48824.

The SON produces the hormones oxytocin and vasopressin. Increased hormone demand, such as occurs during parturition and lactation induces ultrastructural modifications which include: a) increases in soma-somatic appositions, b) dendritic bundling and c) the appearance of new double synapses. Recently observed in maternally behaving virgin females has been an increase in the area of the dendritic zone (Salm et al., Soc. Neurosci. Abstr. 1985, 11, 612). The present study examined the area of the SON dendritic zone for changes in response to stimulus conditions associated with actual motherhood, reexamined dendritic bundling in the entire mediolateral extent of the zone, and investigated the possibility of differential responses within the zone. Ultrastructural analyses were made of the SON dendritic zones from virgin females, prepartum, postpartum, 10 day lactating, and 30 day postlactating rats (n=6 per condition except in prepartum in which n=5). Montages of the entire dendritic zones were constructed, and then area measurements of the proportion of each zone comprised of dendritic profiles, and of the total zone area (the dendritic region, plus the ventral glial lamina; VGL) were obtained. For the purposes of analysis the dendritic region was partitioned into 3 non-overlapping compartments (medial, intermediate, and lateral) of equal size. Photographs of each compartment were used for analysis of the number of dendrites and the extent of dendritic bundling. Data were analyzed with parametric procedures with significance set at $p < 0.05$. Area measurements of the montages revealed increases in the extent of: a) dendritic region, b) VGL, and c) total area of the zone in the postpartum group compared to virgins and prepartum animals. Examination of the dendritic region at higher magnification revealed increases in the proportion of bundled dendrites in the intermediate and lateral compartments in both postpartum and lactating animals as compared to virgins. Furthermore, in all treatments, the lateral compartment contained significantly more dendrites than did the most medial compartment of the zone. The increase in the size of the dendritic portion of the zone in postpartum animals is concomitant with increases in dendritic bundling; a situation in which the dendrites should actually occupy less area. These increases are most likely due to increases in the size of individual dendrites. Taken together these data confirm and further demonstrate the lability of associations among SON dendrites and glia in response to gestation, parturition and lactation. Supported by NIH grants NS 16942 & NS 09140 and by a fellowship from the Medical Scientist Training Program to KGS.

441.5 CNS PLASTICITY AND BEHAVIORAL DEFICIT FOLLOWING PHOTOCHEMICAL INFARCTION OF THE WHISKER CORTICAL BARREL-FIELDS. B.E. Hurwitz, W.D. Dietrich, P.M. McCabe, B.D. Watson* and N. Schneiderman. Depts. of Psychology and Neurology, Univ. of Miami, Coral Gables, FL 33124.

In previous research we have used a noninvasive photochemical reaction to induce thrombosis leading to infarction of the primary whisker cortical barrel-fields in adult rats (cf. Watson, B.D. et al., Annals Neurol., 17:497, 1985). The present study was undertaken to determine whether a behavioral task requiring detection of whisker deflection would be disrupted after sham or unilateral photochemical cortical barrel-field infarction. Food-deprived adult male Wistar rats (N=14) were trained in the dark to produce a right turn in a T-maze apparatus depending upon whether the right whiskers were deflected by a manually delivered probe. Since it was necessary to control for any auditory stimuli produced by the probe, which could also serve as a directional cue, control trials were performed whereby a similar auditory stimulus was delivered manually on the right side but without stimulating the right whiskers. The animals were reinforced for turning left on these control trials. Therefore, the presence or absence of right whisker stimulation served as the only discriminative stimulus the animals could use to determine which way to turn in the T-maze for reinforcement. Unilateral or sham infarction of the left primary whisker cortical barrel-field was induced after an 80% level of correct left and right-turn responses was achieved for 3 successive sessions. Post-infarction testing was performed every other day from the third to the 60th post-infarction day.

The results revealed a significant behavioral deficit in the unilateral animals, with a mean decrement in the percent of correct responses of 27.6% at post-infarction day 3. This deficit was maintained throughout the 60 day period with a slight recovery to the-20% level. These animals significantly differed from the sham-infarcted animals who exhibited a small decrease of 14.2%in the percentage of correct responses at post-infarction day 3 but recovered to pre-infarction levels by day 7 and maintained this level throughout the testing period.

Therefore, as a consequence of unilateral whisker barrel-field infarction, rats were unable to utilize whisker deflection information in the production of a motor response. Current research is assessing the extent to which behavioral recovery can be enhanced with pharmacologic agents. Supported by NINCDS (NS18749).

441.6 ALTERED PATTERNS OF CEREBRAL CORTICAL LATERALIZATION IN MALE RATS WITH INCREASED LEVELS OF ENVIRONMENTAL COMPLEXITY. J. C. Lin*, E. R. Greer*, M. C. Diamond. Department of Physiology-Anatomy, University of California, Berkeley, CA 94720.

In general, the right cerebral cortex of the male, Long-Evans rat is thicker than the left in animals living in standard colony conditions (3 rats/small cage, 32x20x20 cm) (Diamond, M. C., Cerebral Dominance, Eds. Geschwind, N. and Galaburda, A. M., Harvard Univ Press, 1984). In recent experiments with enrichment (12 rats/large cage, 70x70x46 cm plus "toys", changed twice weekly) and/or a high population density (36 rats/large cage), two left cortical areas became consistently thicker than the right, thus reversing the right dominance pattern. Transverse, frozen, 40 mu sections were cut from the frontal, somatosensory and occipital cortex and stained with a modified thionine stain. From microslide projected images, cortical thickness measurements were taken on the brains from animals living in their various environments from 60-66 to 90-97 days of age. Area 18, a visual association, medial-occipital cortical region, showed significant left dominance (1-3%, $p < 0.05$-.01)in those animals living in enriched and enriched/high population density. In adjacent area 17, primary visual cortex, the usual right dominance pattern was eliminated. In area 3, a general sensory region, the left cortex was thicker than the right by 2-3% ($p<0.025$-.005) in the same two living groups. In other words, the enriched and enriched/high population density experiments altered laterality patterns. The significance of these changes is yet to be determined.

441.7 THE INFLUENCE OF TEMPERATURE VARIATION ON ACTIVITY-ASSOCIATED ULTRASTRUCTURAL PLASTICITY IN TETANICALLY STIMULATED NODES OF RANVIER. C. Wurtz and M. Ellisman (SPON: J. Linder). Lab. for Neurocytology, Dept. Neurosciences, UCSD, La Jolla, CA 92093.

We have previously reported a change in morphology of the amphibian node of Ranvier that is associated with repetitive action potential propagation (Wurtz & Ellisman, J. Neurosci. 6:11, 1986). This change appears as an increase in the size and number of extracellular vacuoles within the paranodal apparatus, it is reversible and is seen regardless of the method of fixation employed. The morphological alteration is closely correlated with a reduction in the conduction velocity, and a depression of the amplitude of the compound action potential (CAP). Such perturbations of CAP conduction parameters are like those seen in intermittent conduction of myelinated axons (Barron & Matthews, J. Physiol. 73:85, 1935). Utilizing a hybrid physiological - morphological experimental preparation, we have now assessed both the conduction properties of the CAP and the associated structural changes in the node over a range of temperatures. We find that CAPs recorded from intact dorsal roots exhibit normal physiology at 32^{0} and can be activated for extended periods with much lower vacuolization than occurs at temperatures below 22^{0}. Reducing the temperature to 7^{0}, however, causes a substantial depression in CAP conduction properties which is associated with a degree of paranodal disruption far in excess of that noted at 17^{0}. Post-stimulation recovery is also influenced by the temperature of the preparation. In these cases stimulation at 7^{0} followed by recovery at 7^{0} completely prevented the paranodes from reobtaining control morphology, if however the bath was warmed to 17^{0}, nodes were able to partially recover. These results are in accordance with those of the early physiologists on whole-nerve conduction, and suggest that impaired CAP amplitudes, conduction velocities, and increased vacuolization which occur at low temperature may be due to the suppression of temperature sensitive metabolic activities.

Finally, data obtained from voltage clamped amphibian nodes have been held to be non-extrapolatable to mammalian processes because such nodes will not produce action potentials above 30^{0}. However we note that intact fiber bundles conduct at 32^{0} with an enhanced efficacy. We speculate that this disparity in temperature effects between dissected and intact nerves may be due to partial disruption of a structural complex such as the reticulum of extracellular fibrils which are found connecting structures within the nodal gap to the extracellular matrix. Damage to such delicate structures would be unavoidable given the methods for single fiber dissection.

441.8 RECOVERY OF FOREPAW USAGE AFTER MEDIAN NERVE CRUSH IN THE TWITCHER HETEROZYGOTE. S. D. Lavine* & Ch. E. Olmstead (SPON: G. Galbraith) UCLA/MRRC Research Group, Lanterman Developmental Center, Pomona, CA 91769

We have previously reported that mice heterozygous for the twitcher mutation were slower to recover from a sciatic nerve nerve crush than normal littermates. The current study reports the recovery of paw usage on a pellet grasp task following a crush of the median nerve.

The subjects were adult male C57Bl/6j-twi mice, normal (N = 10) or heterozygous (N = 8) for the twitcher mutation reared in our laboratory. Under Nembutal anesthesia the median nerve was exposed and crushed for 60s with a pair of surgical forceps.

Pellet Grasp Task. The apparatus consisted of a clear acrylic box 30cm X 20cm X 15cm with a slot (5cm high and 0.5cm wide) 1.0cm from the floor on each side of the movable front panel. The animals, food deprived to 85-80% of their normal body weight, were trained to reach through the slot and grasp 20mg food pellets balanced on a 6mm diameter glass rod placed ~1cm from the front of the cage. Pellets were continuously replaced by the experimenter as the mouse either grasped or knocked the pellet from the rod. Successful trials were scored as a 'Grasp', 'Knock In'(when the pellet was batted onto the cage floor) or 'Knock Out' when the mouse merely knocked it off of the rod. Twenty six sessions were run at biweekly intervals prior to surgery and resumed 18 days post-operatively.

Neurological Testing. Beginning 1 day post-operatively, limb use was evaluated by a neurological battery consisting of paw posture, contact placing responses, drag grasp, climb grasp, and hang grasp. Recordings of footprint pattern were made using the stamp pad technique. The experimenter was blind to the individual genotypes throughout the tests.

Prior to surgery all animals reached stable limb preferences by the 6th session. A bias index showed that the normal animals had stronger side preferences than the heterozygotes. Although there was not a significant difference in the number of pellets grasped, the heterozygotes showed significantly more attempts and failures than the normals. There were no neurological differences between genotypes prior to surgery. Post-operatively, the neurological battery showed slightly faster recovery in the normal animals (Mean = 19.6 days) versus the heterozygotes (Mean = 20.3 days). The range in the heterozygotes was somewhat larger. Both groups retained their pre-operative preference, but showed slower performances on the grasp task with some suggestive differences between genotypes.

441.9 FURTHER ANALYSIS OF THE DISRUPTIVE EFFECTS OF CHRONIC DIAZEPAM ON RECOVERY OF FUNCTION AFTER BRAIN DAMAGE. T.D. Hernandez, J. Kiefel*, M.D. Lindner*, G. Knight* and T. Schallert. Dept. of Psychology and Institute for Neurol. Sci., Univ. Texas, Austin, TX 78712.

A regimen of certain anticonvulsant drugs can severely and chronically disrupt recovery from behavioral asymmetries caused by unilateral neocortical damage (Watson, C.W. & M.A. Kennard, *J. Neurophysiol.* 8: 221, 1945; T. Schallert et al., *Brain Res.*, 379: 104, 1986; S. Brailowski et al. *Brain Res.*,1986). To determine whether there might be a sensitive postoperative period during which recovery is especially vulnerable, the postoperative onset of a 7-day regimen of diazepam (or vehicle control) was varied systematically in rats with unilateral lesions of the anterior medial cortex (or sham operations). Behavioral asymmetries were assessed in detail and quantified using somatosensory extinction procedures (described in Schallert et al., 1987), and forelimb placing tests.

Diazepam interfered with recovery from asymmetry recorded in the somatosensory extinction tests. Under the conditions of this experiment there appeared to be a definable period of sensitivity to diazepam extending up to 120 hrs after the damage. Variability in the duration of this period may reflect individual differences in the onset of behavioral recovery.

Diazepam did not affect recovery from the forelimb placing deficits. Perhaps restoration of normal placing reactions depends significantly on motor learning, which may be one of several factors that can limit susceptability to the detrimental effects of diazepam.

Suggested mechanisms and data from several ongoing experiments will be discussed, including the effects of co-administration of diazepam and benzodiazepine antagonists, potential beneficial effects of GABAergic antagonists (at convulsant vs non-convulsant doses), or the effects of the glucocorticoid dexamethasone on recovery from unilateral damage to sensorimotor cortex.

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441.10 REORGANIZATION OF THE SEROTONERGIC PLEXUS OF THE AREA DENTATA AFTER LESIONS OF THE MEDIAN RAPHE NUCLEUS. J.H. Haring. Department of Anatomy and Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

The serotonergic (5HT) plexus of the area dentata (AD) arises from neurons of the median (MRN) and dorsal (DRN) raphe nuclei. The projections of the MRN and DRN are topographically distributed in the AD, with the DRN primarily supplying the molecular layer and the MRN terminating mainly in the hilar region (Azmitia, E.C. and Segal, M., J. Comp. Neurol. 179:641, 1978). Lesions of the cingulum bundle induce the sprouting of 5HT fibers in the hippocampus which results in the reestablishment of normal 5HT plexus in the hippocampal formation (Zhou, F.C. and Azmitia, E.C., Brain Res. 373:337, 1986). This report presents light microscopic observations which demonstrate that indolamine - specific neurotoxin lesions of the MRN induce a proliferation and migration of 5HT axons resulting in a modified 5HT plexus in the area dentata.

Adult, male Sprague-Dawley rats anesthetized with sodium pentobarbital received stereotaxic injections of 5,7 dihydroxytryptamine in the MRN. The AD of these animals was studied using 5HT immunocytochemistry. Two weeks after MRN lesion, the 5HT plexus of the molecular layer is normal, but the 5HT innervation of the hilar region is greatly diminished. Six weeks after lesion, the density of the hilar 5HT innervation remains less than normal, but a proliferation of 5HT fibers is seen in the molecular layer immediately adjacent to the granule cells. Not only do the 5HT fibers seem to be more numerous, but the caliber of these axons appears to be greater as well. Reactive 5HT axons also project from the inner molecular layer through the granule cell layer to the hilar region where they ramify to reinnervate the hilar region immediately adjacent to the granule cells. Eight weeks post-lesion, the 5HT plexus is essentially normal although some enlarged varicosities are still seen. No large reactive fibers are present, and the distribution of the 5HT plexus is comparable to that of control cases. At 12 weeks, the morphology of hilar 5HT axons is generally normal, but bulbous, intensely 5HT immunopositive structures similar to degenerating axons observed 2 weeks post-lesion are seen in the molecular layer. By 20 weeks, the distribution of 5HT fibers and boutons in the hilar region is normal, but the density is still somewhat diminished. By contrast, the molecular layer is almost devoid of 5HT fibers. These observations suggest a shift in the terminal distribution of 5HT fibers from the DRN after MRN lesion. That the hilar region is preferentially reinnervated at the expense of the molecular layer further suggests the existence of a special relationship between hilar neurons, particularly basket cells, and the 5HT input to the AD.

441.11 ALTERATION OF FUNCTIONAL REPRESENTATIONS OF VIBRISSAE IN THE BARREL FIELD OF ADULT RATS. J.T. Yun*, M. M. Merzenich and T. Woodruff*, Coleman Laboratory, University of California at San Francisco, San Francisco, CA 94143.

Detailed microelectrode maps of the SI vibrissal zone were derived in a series of normal adult rats in which the tips of subsets of vibrissae were coupled to promote their predominantly simultaneous stimulation. Studies were designed to explore the possibility that the normal sequenced, non-simultaneous stimulation of vibrissae accounts, in part, for their functional representational segregation.

Several days before each electrophysiological mapping experiment, the tips of one or more small groups of vibrissae (usually pairs) were coupled together by the application of a thin layer of methyl cyanoacrylate gel. The remaining vibrissae were clipped in some rats; they were left intact in others. In most experiments in this series, one investigator prepared the rats and clipped all vibrissae to an equal length just prior to the mapping experiments; the other two were blind to the identity of the long and coupled vibrissae. Blind control experiments were also conducted; in these studies, all vibrissae were left intact and undisturbed. All experiments were conducted in ketamine-anesthetized rats. Between about 100 and 150 microelectrode vertical, parallel penetrations were introduced into the vibrissal representational zone in most experiments. Vibrissae effective for evoking a response of multiunit clusters were defined by their careful manual manipulation with fine glass probes. As judged by penetration depths, receptive fields were defined within granular and supragranular cortical layers.

In most but not all cases, there was a substantial enlargement of the functional cortical representations of long vibrissae of rats maintained in a tactually rich environment after all other vibrissae have been trimmed for only a few days. There was also a higher probability that coupled vibrissae would be represented together at sampled cortical loci. To site a representative example, in an adult rat in which most of the vibrissal representation was explored in a 137-penetration map 8 days after whiskers were glued and trimmed, neurons at 29 cortical sites responded to stimulation of both vibrissae constituting the long glued pair (D2-D3; other vibrissae were trimmed in this rat). At 13 cortical locations, neurons were driven exclusively by stimulation of the two whiskers consituting the long glued pair. Stimulation of other combinations of vibrissae never evoked responses at more than a few locations. In other rats in which the D2 and D3 region was mapped in equal detail and in which these whiskers were not long and/or glued --- and in control rats --- these whiskers were conjointly effective for driving neurons at not more than a few cortical sites.

The barrel fields of these rats are being histologically reconstructed so that they can be related to these detailed cortical maps. Results up to this time indicate that a few days of differential behavioral stimulation of a small vibrissal subset may result in a mismatch between the anatomically constant layer 4 barrel array and the physiologically manifested territories of representation of the vibrissae in these adult rats.

These results support the hypothesis derived in earlier studies in monkeys that, considered in detail, cortical representations and receptive fields are shaped by differences in the magnitudes of inputs, and by the time structure of inputs.

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441.12 REPETITIVE INTRACORTICAL MICROSTIMULATION ALTERS THE AREA 4 REPRESENTATION OF MOVEMENTS. R.J. Nudo and M.M. Merzenich. Coleman Laboratory, Departments of Physiology and Otolaryngology, University of California at San Francisco, San Francisco, CA 94143.

During the past two decades, intracortical microstimulation (ICMS) has been used extensively to determine the functional organization of motor cortex in a number of mammals. Large sources of variability in the movements evoked by ICMS are not uncommon, as have been discussed by previous investigators (e.g., variability among individuals, with levels of anesthesia, etc.). The present study was designed to determine whether electrical stimulation of the motor cortex itself modifies subsequent evoked motoric responses, as suggested by Graham Brown and Sherrington in 1912. We asked two specific questions: 1) Does repetitive ICMS in primary motor cortex (MI) change the organization of the functionally defined motor map? 2) If so, is feedback from the periphery requisite for effecting those representational changes?

Experiments were conducted in adult, ketamine-anesthetized rats using conventional ICMS techniques. Microelectrode penetrations were sited with reference to the surface vasculature, then advanced perpendicular to the cortical surface to a depth of 1700 microns. At each site, the movement evoked by stimulation at near-threshold current levels was determined.

In these motor maps, borders separating movements of the forelimb and the vibrissae, the forelimb and the neck, and the forelimb and the hindlimb were defined in great detail (i.e, with about 100 micron interpenetration distances). A microelectrode was then introduced near one of these defined borders, and 40 msec long, 300 pps stimulus bursts then were delivered at a rate of 1/sec for one to three hours. Monophasic stimuli were charge-balanced. Two stimulus levels were used in different preparations in this experimental series. In one group, the current level was 2X the threshold current for evoking the site-specific movement (suprathreshold group). In the second group, the current level was .33 to .5X the movement-threshold current (subthreshold group).

With repetitive stimulation on this schedule, defined representational borders shifted significantly (up to 500 microns) in both suprathreshold and subthreshold stimulation experiments. The specific movement evoked by stimulation at the heavily stimulated site thereby came to be represented over a substantially larger cortical territory. Territorial expansion appeared to be progressive. Boundaries slowly reverted to near their original positions during a one to three hour rest period following repetitive ICMS. In the subthreshold group, changes were observed after repetitive stimulation at current levels well below those required to evoke movements. No electromyographic activity could be detected at these current levels. Thus, observed changes were apparently not dependent upon afferent feedback from the periphery.

Recent evidence has demonstrated that somatosensory cortical fields in adult mammals undergo significant functional reorganization as a result of a number of different types of manipulation. The present results indicate that the organization of motor maps of area 4, like that of somatosensory maps, is subject to modification in adult mammals. As concluded earlier for surface stimulation by Graham Brown and Sherrington, these studies indicate that the results of ICMS experiments can to some extent be shaped by applied cortical stimulation. Further, they reveal that, at least under these unusual experimental circumstances, large representational changes can be generated very rapidly within area 4 in the absence of any evident peripheral feedback.

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441.13 CEREBELLAR PLASTICITY: PRELIMINARY EVIDENCE THAT LEARNING, RATHER THAN REPETITIVE MOTOR EXERCISE, ALTERS CEREBELLAR CORTEX THICKNESS IN MIDDLE-AGED RATS. J.E. Black, A.L. Jones*, B.J. Anderson, K.R. Isaacs, A.A. Alcantara, & W.T. Greenough. Coll. Med., Depts. Psychol., Anat. Sci., & Neur. & Behav. Biol. Prog., University of Illinois, Champaign, IL 61820.

Previous demonstrations that experience can alter dendritic morphology in cerebellar cortex have left open the issue of whether motor learning or repetitive motor exercise is primarily responsible (Pysh & Weiss, 1979, Science, 206, 230; Floeter & Greenough, 1979, Science, 206, 227). Thirty-eight 11-month-old Long-Evans hooded female rats were randomly assigned to one of four experimental conditions balanced for litter: Individual Condition (IC), in cages, Acrobatic Condition (AC), with extensive visuomotor training, Forced Exercise (FX), with a treadmill exercise regime, Voluntary Exercise (VX), with free access to a running wheel. Body weight, food consumption, and estrous cycling were assessed for all animals. Muscle enzymes were assayed to provide a measure of physical exercise. IC and VX rats were handled daily during the 30 days of the experiment. AC rats were trained to traverse an obstacle course for five trials per day that became progressively more difficult over the 30 day period. The path did not branch, hence it was not a spatial maze, and required the rats to traverse obstacles such as narrow balance beams, swinging bridges, ropes and chains, and teeter-totters. The FX rats were given progressively longer endurance training on a treadmill at 10 m/min until the daily sessions were 60 minutes long. VX rats had 24-hour access to a running wheel attached to their cages for the duration of the study. IC rats were housed individually in standard laboratory cages. The rats were anesthetized, perfused with mixed aldehydes, and dissected. Heart, liver, stomach, adrenals, ovaries, and spleen were examined to assess relative levels of physiological function. Blocks from cerebellum, visual, and sensory-motor cortex were prepared for light and electron microscopy. One-micron semithin sections of cerebellar folia were drawn at 100X, and molecular layer thickness was measured at 200 micron intervals along the pial surface. The molecular layer in AC rats was approximately 6% thicker than in the other three groups, $F=5.61$, $p<.05$. The amount of wheel running and cerebellar cortical thickness were not correlated in the VX rats (Spearman $r=-.21$, n.s.), also indicating that motor exercise alone does not account for the cerebellar effects.

MEAN THICKNESS OF MOLECULAR LAYER (microns), VERMIS + LATERAL FOLIA COMBINED:

IC	VX	FX	AC	p (AC vs. others)
227	230	230	243	<.05

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441.14 DIFFERENTIAL PLASTICITY BETWEEN TWO SUBTYPES OF PYRAMIDAL CELLS IN THE MOTOR-SENSORY FORELIMB CORTEX AS A CONSEQUENCE OF REACH TRAINING. G.S.Withers and W.T.Greenough. Depts. of Psychol., Anat. Sci., and the Neural & Beh. Biol. Program, University of Illinois, Champaign, IL 61820

Apical dendrites of layer V pyramidal cells in a region of Motor-Sensory Forelimb cortex (MSF) were previously found to be altered in rats trained to reach (Greenough, Larson, and Withers, Beh.& Neural Biol, 44, 301-14, 1985). Young adult rats were trained to reach into a tube for bits of cookies with either their preferred or nonpreferred paw (33 reach sessions over 16 days). Analysis of Golgi-impregnated tissue indicated that total dendritic length and number of branches were greater in the MSF contralateral to the trained paw than in the hemisphere opposite the nontrained paw. These within-animal differences were greatest in rats trained to reach with their nonpreferred paw. This study investigates subtypes of another cell population within this region. The MSF sample region was the area of highest uptake of 2-DG in animals which were injected prior to performance of the reaching task (Fuchs, et al, Soc. Neurosci. Abstr., 9, 54, 1983). Basilar branches of pyramidal cells in layers II/III were traced using a computerized tracking system. The pyramidal cells were divided into two subtypes based upon morphological differences; the first type having a forked apical dendritic shaft, the second type having a single primary apical shaft. We observed that the "forked" subtype of cells are typically located higher in the cortex than are the single shaft cells, suggesting that these cells may be primarily layer II cells. The data presented below compare all of the "trained" hemispheres with all of the "nontrained" hemispheres. Significant differences were found in the total dendritic length (TDL) (in microns) and number of dendritic intersections with concentric spheres for the forked cells, whereas no significant differences were found in the single shaft cells. These results may suggest differential input to these two subtypes of cells, or may indicate differential sensitivity to training between these two subtypes.

	FORKED CELLS		SINGLE SHAFT	
	TRAINED	NONTRAINED	TRAINED	NONTRAINED
TDL	1471	1272 p<.02	1233	1159 n.s.
# BRANCHES	34	30 n.s.	29	27 n.s.
INTERSECTIONS	133	114 p<.01	111	106 n.s.

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441.15 **PLASTICITY OF INNERVATION OF SUPERIOR COLLICULUS BY RETINAL TRANSPLANTS.** J.D. Radel[1], L. Galli*[2] and R.D. Lund[1].
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Retinal ganglion cells innervate the most superficial 400 - 500 μm of the contralateral superior colliculus (the superficial gray and the optic laminae) in normal rats. In the presence of this normal optic input, embryonic retinae transplanted to the dorsal surface of the midbrain in neonatal rats survive, differentiate and innervate the superior colliculus with a projection restricted to its dorsal surface. We have examined how this pattern of innervation, which is established by one month of age, can be modified by depriving the superior colliculus of its normal optic input.

Embryonic (E14) rat retinae were transplanted above one superior colliculus of neonatal (P1) rats. Experimental animals received a unilateral enucleation at 30 days of age, and at 60 days the transplanted retinae were exposed and either injected with HRP or lesioned by aspiration. In control animals, with both eyes intact, the transplant was injected with HRP either 30 or 60 days after transplantation. The host was perfused 24 hours after HRP application or 72 hours after lesioning and the midbrain tissue processed for either light or electron microscopy. For HRP-injected transplants, coronal sections of the brain were cut at 40 μm and reacted by the TMB method or at 70 μm, with alternate sections reacted by the TMB method for light microscopy and the nickel-intensified DAB reaction for electron microscopy. For lesioned transplants, tissue from both the left and right superior colliculus was processed for electron microscopy.

The enucleation resulted in substantial innervation of the superficial gray lamina by transplant axons. These axons possessed elaborate arborizations, resulting in a fine, granular projection throughout this lamina. Electron microscopic analysis revealed that the superficial gray lamina, the normal termination zone for retinal ganglion cells, contained many transplant-derived pre-synaptic profiles. Fibers were also occasionally seen projecting to the contralateral superior colliculus, in which the normal visual projection remained intact. These fibers, as in controls, were confined to the surface of the colliculus, and transplant-derived pre-synaptic profiles were similarly restricted.

These results indicate that depriving the host superior colliculus of its normal optic input in the presence of fibers from transplanted retinae induces sprouting of transplant fibers into the denervated terminal space. This sprouting of existing transplant fibers, and the subsequent formation of synapses in the tectum, demonstrates that established projection from retinal transplants can alter their patterns of innervation in mature animals.

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441.16 INDUCTION OF c-fos PROTEIN IN NEURONS OF THE CENTRAL NERVOUS SYSTEM. S.P. Hunt [1]*, A. Pini [2]*, M.L. Errington [3]*, T.V.P. Bliss [3]*, G. Evan [4]*, (SPON: D. Bousfield). (1) MRC, Molecular Neurobiology Unit, Cambridge, CB2 2QH, UK. (2) Dept. Pharmacology, Univ. Cambridge CB2 2QD, UK. (3) NIMR, Mill Hill, London, NW7, UK. and (4) Ludwig Institute, Cambridge, CB2 2QH, UK.

We have investigated the conditions under which the proto-oncogene c-fos is expressed in neurons of the rat spinal cord and hippocampus. Because of the suggested link between the expression of the c-fos proto-oncogene and prolonged changes in excitable cells (Goelet, P. et al. Nature 322; 419, 1986), we looked for evidence that the c-fos protein is induced in spinal cord neurons following sensory stimulation, as sensory stimuli which actuate small diameter afferents produce long term changes in dorsal horn neurons (Wall, P.D., Phil. Trans. Roy. Soc. B. (Lond) 308: 393, 1985). All experimental procedures were performed in anaesthetised S.D. rats (Equithesin 4ml/kg i.p.). 20 min to 4 hr following sensory or electrical nerve stimulation animals were perfused with 4% paraformaldehyde and tissue processed for immunocytochemistry. Immunoreactivity was observed within nuclei of dorsal horn neurons using immunoperoxidase histochemistry and antisera directed against the p55 c-fos protein. Immunoreactive neurons were observed predominantly in spinal cord laminae I, II, V and X 20 min - 2 h following cutaneous application of mustard oil (5%) or radiant heat (52°C). However, following non-noxious stimulation, p55 c-fos positive nuclei were observed mainly in laminae II to IV. Injection of mustard oil into the medial gastrocnemius muscle resulted in the appearance of labelled nuclei within laminae I, V-VII and X. Expression of the proto-oncogene was not seen in regions of the spinal cord such as n. gracilis or ventral horn which receive direct inputs from low threshold large diameter afferents.

In contrast, 1-2 h following stimulation of the perforant pathway (250 Hz, 200 ms, 3 trains) sufficient to generate long term potentiation in dentate gyrus, no induction of p55 c-fos was observed. However direct perfusion of the dentate gyrus with 10 mM calcium, 10 μm carbachol or electrical stimulation of CA3 did result in a massive expression of the proto-oncogene within ipsilateral dentate granule cells.

Thus p55 c-fos can be induced in neurons of the CNS by a variety of stimuli, but synaptic induction of the gene has only been observed in the spinal cord where it may indeed be related to synaptic reorganisation

441.17 DISTRIBUTION AND INDUCTION OF c-fos PHOSPHOPROTEIN IN ADULT MAMMALIAN BRAIN. M. Dragunow and H. A. Robertson, Department of Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

Using immunohistochemical techniques we have recently shown that the product of the c-fos protooncogene, the c-fos phosphoprotein, is present in the adult mammalian brain (Dragunow et al, Eur. J. Pharmacol., 135:113, 1987). Furthermore, the immunostaining is found predominantly within the nuclei of nerve cells in distinct cortical layers, hippocampal pyramidal cells, dentate granule cells and cells in the striatum, amygdala, entorhinal and piriform cortices and granule and purkinje cells in the cerebellum. Axons and dendrites of nerve cells are not stained. Pre-absorbing the c-fos anti-serum with the peptide that it was raised against abolished the immunostaining as did ommision of anti-fos serum.

Here we report that high frequency unilateral electrical stimulation of the dorsal hippocampus which induces partial seizures and leads to a permanent increase in the response of the brain to future seizures (ie: kindling), produces a large, selective and rapid but transient increase in c-fos protein-like immunoreactivity bilaterally in granule cells of the rat dentate gyrus. At 10 min following the termination of the seizure the number of granule cells showing positive immunostaining is not different from basal levels. However, at 30 min, 60 min, and 4 hours post-seizure the number of granule cells showing positive immunostaining is greatly increased from basal levels. Twenty-fours after the seizure c-fos protein-like immunoreactivity has returned to basal levels. In rats electrically stimulated (60 Hz, 500 uA peak-to-peak, 0.5msec biphasic pulses for 2 sec) but treated with carbamazepine (40 mg/kg, i.p.) to prevent seizures a small increase in immunostaining, ipsilateral to the side of stimulation, is observed 1 hour later. Generalized seizures evoked in hippocampal kindled rats are also associated with a large increase in immunostaining in dentate granule cells. However, seizures evoked by injection of pentylenetetrazol (50 mg/kg, i.p.) do not lead to an increase in immunostaining in dentate granule cells, but are associated with increased immunostaining in cells in the piriform cortex.

The presence of c-fos protein-like immunoreactivity in the mature mammalian brain, together with its rapid induction during electrically-induced seizures and high frequency electrical stimulation suggest that the c-fos protooncogene is involved in plastic changes in the mature nervous system.

We thank the Medical Research council of Canada for support and Dr. Tom. Curran (Roche) for providing anti-fos serum.

441.18 LOCALIZATION OF GAP-43 (B-50) IN THE RAT BRAIN. L.I. Benowitz, S.P. Finklestein, P.J. Apostolides, N.I. Perrone-Bizzozero and H. Zwiers. Depts. of Psychiatry, Neurology, and Program in Neuroscience, Harvard Med. Sch.; Mailman Res. Ctr., McLean Hosp., Belmont, MA 02178; Neurology Unit, Massachusetts General Hosp.; Dept. of Pharmacology and Therapeutics, Univ. of Calgary Med. Sch.

GAP-43 (GAP-48, B-50, F1, pp46) is a phosphoprotein of the presynaptic membrane that is expressed at high levels when neurons are establishing synaptic relationships. Although most neurons cease expressing GAP-43 after their connections have become stabilized, considerable levels persist in certain parts of the brain throughout life, and may be important in mediating ongoing synaptic plasticity in these areas. In the rat hippocampal formation, the phosphorylation of this protein (F1) by kinase C has been linked with the events that underlie long-term synaptic potentiation. To gain further insight into the relationship between GAP-43 and brain functioning, we used two different anti-GAP/B-50 antibodies to map the distribution of the protein in the brains of 8 adult rats. One antibody was raised in sheep against electrophoretically pure GAP/B-50, the other in rabbit against a pure protein and then affinity purified on a B-50 column. Both antibodies reacted uniquely against GAP/B-50 on Western blots, and both gave identical patterns of immunostaining in brain tissue. GAP/B-50 immunostaining was restricted almost entirely to the neuropil, and showed a generally increasing caudorostral gradient along the neuraxis. Immunoreactivity was highest in layer I of cortex, the CA1 field of the hippocampus, and in a continuum of subcortical structures that included the caudate-putamen, nu. accumbens, olfactory tubercle, substantia innominata, bed nucleus of the stria terminalis and parts of the amygdala, medial preoptic area and medial hypothalamus. Essentially no staining at all was seen in somatosensory and somatomotor relay areas of the brainstem. Striking differences were also noted in the staining of various nuclei of the dorsal thalamus and of different cytoarchitectonic areas of cortex. This distributional pattern raises the question of whether synapses in all areas containing high levels of GAP-43/B-50 are capable of undergoing functional plasticity, or whether the protein may function in at least some of these areas in some other capacity (e.g., general signal transduction).

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441.19 THE GENE ENCODING THE GROWTH-ASSOCIATED PROTEIN GAP-43 (B-50, F1) IS EXPRESSED SELECTIVELY IN ASSOCIATIVE AREAS OF THE HUMAN NEOCORTEX. R.L. Neve, S.P. Finklestein, E.D. Bird and L.I. Benowitz. Depts. of Pediatrics, Neurology, Neuropathology, Psychiatry, and Program in Neuroscience, Harvard Med. Sch.; Dept. of Genetics, Children's Hospital; Neurology Service, Massachusetts General Hosp.; Mailman Res. Ctr., McLean Hosp., Boston and Belmont, MA.

The growth-associated protein GAP-43, a protein that has been associated with the development of synaptic relationships, has recently been shown to be equivalent to phosphoprotein B-50 (F1), a C-kinase substrate which has been implicated in phosphoinositide metabolism and in the events underlying long-term synaptic potentiation. Thus, although most neurons cease expressing GAP-43 at high levels after the establishment of stable synaptic relationships, its continued expression in a select subset of neurons may confer specialized properties upon these cells' synaptic endings, perhaps related to ongoing functional plasticity. To identify such areas in the human brain, we used a full-length cDNA probe to the human gene (Neve et al., Mol. Brain Res., in press) in Northern blots to examine levels of GAP mRNA in different areas of embryonic and adult human brains. In tissue from 19- and 22-week fetuses, levels of the 1.6 kb GAP mRNA were extremely high in all brain regions examined, consistent with the idea that all neurons express high levels of the protein when synaptic relationships are being established. In four adult brains, however, levels of GAP mRNA showed a highly reproducible, striking regional heterogeneity. Levels of GAP mRNA were extremely low in thalamus, caudate-putamen, and in sensory and motor areas of the neocortex (e.g., Brodman Areas 17, 18 and 4). However, higher integrative areas of the cortex showed very high levels of GAP mRNA, including the inferior temporal region (Areas 20,21), frontal cortex (Areas 10 and 11) and perisylvian structures (Areas 40 and 44). Levels of GAP mRNA were modest in the cerebellum and low in the hippocampus as a whole, although preliminary in situ hybridization studies suggest that specific neural populations in the hippocampus do express the gene. Controls included use of other genetic probes (e.g., cDNAs for microtubule associated proteins and glyceraldehyde 3-phosphate dehydrogenase) that showed equal distribution in all areas. These results suggest that, by virtue of their continued expression of the GAP gene, a subset of neurons in associative areas of the brain may be specialized for ongoing synaptic plasticity.

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441.20 THE EFFECT OF AXOTOMY AND DEAFFERENTATION ON PHOSPHORYLATION DEPENDENT ANTIGENICITY OF NEUROFILAMENTS IN RAT SUPERIOR CERVICAL GANGLION NEURONS. (SPON: B. Cooper). G. Shaw*, D. Winialski* and P. Reier. University of Florida College of Medicine, JHM Health Center Box J-244, Gainesville Florida 32610.

Previous immunocytochemical studies have shown distinct immunological differences between the neurofilaments present in axons and those of dendrites and perikarya in many mature neuron types. These differences are now known to be at least partly due to local differences in the levels of phosphorylation of the neurofilament subunit proteins. In a recent study we found that such immunological differences are also present in normal rat superior cervical ganglion neurons (SCG). Furthermore, we observed that axotomy, performed by cutting the postganglionic fibres, results in the rapid induction of phosphorylated neurofilaments in the dendrites and perikarya of some SCG neurons, as has been observed in other cell types. We have also observed that preganglionic section causes similar changes in the levels of phosphorylation in the neurofilaments in many neuron cell bodies, so that phosphorylated epitopes become visible in the cell bodies of presumptively denervated but otherwise undamaged neurons. The changes in this case occur, however, over a longer time period than those induced by postganglionic lesions. In addition, these changes are accompanied by the induction of staining with some neurofilament antibodies in the nuclei of the neurons. The acquisition of staining by both pre- and postganglionic lesioned animals proved to be reversible, so that after 9 - 11 days no neurons in either experimental group exhibited dendritic/perikaryal phosphorylated epitopes. These results indicate that changes in the level of neurofilament phosphorylation are not only inducible by damage to the cell, but also apparently by deafferentation. Supported by NIH grants NS 22695 (GS) and NS 22316 (PJR).

DEVELOPMENTAL DISORDERS

442.1 **AMPHETAMINE-INDUCED BEHAVIOR IN RATS TREATED WITH PHENYLACETATE PERINATALLY.** D.L. Dow-Edwards, A. Rabe and L.J. Porrino. (Spon: E. Elowitz) Lab of Cerebral Metabolism, Dept of Neurosurgery, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203; New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314; and Addiction Research Center, National Institute on Drug Abuse, Baltimore, MD 21224

Phenylacetate (PA), a major metabolite of phenylalanine, may be the primary cause of brain dysfunction associated with sustained hyperphenylalanemia. Biochemical (Loo et al., Life Sci. 27:1283, 1980), morphological (Robain et al., Acta Neuropath. 61:313, 1981), and behavioral (Fulton et al. Life Sci. 27:1271, 1980; Rabe et al. Soc. Neurosci. 360.13, 1983; Loo et al. Dev. Neurosci. 6:227, 1984; Rabe et al. Soc. Neurosci. 120.6, 1985) evidence to support this hypothesis has appeared. Behavioral responses to amphetamine are well established. Low doses produce locomotion and high doses stereotypy. The locomotor responses have been associated with the mesolimbic system and the stereotypic responses with the nigrostriatal system. Therefore we used amphetamine to identify whether specific neuronal systems might be altered by perinatal PA administration.

Sprague-Dawley rats were time mated and housed under standard conditions. One group of animals was infused with PA at 7uMol/g/20ml/24hr between gestation day 9 and 20 via a chronic subdermal catheter (Pre). A control group received the identical volume of saline (Sal). On the day of birth (PD 1) litters were culled to 8. Other litters from untreated dams received PA at 2.5-3.5 uMol/g twice daily between PD 2 and 22 (Post). A fourth group which received prenatal PA also received 1.5-2.0 uMol/g body weight twice daily between PD 3 and 8 (Pre+Post). All animals were weaned at PD22. At 6.5-7 months of age, all animals were randomly assigned to receive either vehicle, 1 or 5 mg/kg amphetamine sulfate ip. Animals were held individually in the home cage for 45 minutes and then placed in a 30.5 X 30.5 sq inch open field. The following behaviors were manually recorded by individuals unaware of the experimental treatment of the rat: locomotion, rearing, and stereotypy.

The results show that the low dose of amphetamine induces locomotion and the high dose induces stereotypy in all groups ($p<0.05$, ANOVA). However, PA administration altered the locomotor and rearing responses to amphetamine ($p<0.05$, ANOVA). Stereotypy, on the other hand, was not significantly affected by the perinatal treatments. Therefore, PA administration during a critical period of development appears to affect the mesolimbic dopaminergic system to a greater degree than the nigrostriatal pathway.

442.2 VALINE, ISOLEUCINE, LEUCINE (VIL): A NEW TREATMENT FOR PKU. H.K. Berry, R.L. Brunner*, M. M. Hunt*, and P. P. White*. Metabolic Disease Center, Children's Hospital Medical Center, Cincinnati, OH 45229.

Phenylketonuria (PKU) is an inherited biochemical disorder in which phenylalanine hydroxylase, the enzyme responsible for conversion of phenylalanine (PHE) to tryosine, is absent or drastically reduced. There is no known cure for the enzyme defect. Left untreated PKU results in progressive mental retardation and measured IQ below 50. The only treatment is severe dietary restriction of PHE beginning early in life.

Recent reports suggest that adverse behavioral changes and impaired performance are consequences of elevated serum PHE even in early treated PKU subjects, whether from deliberate termination of diet or from chronic failure to adhere to the strict regimen. We reported earlier that administration of valine, isoleucine, and leucine (VIL) to PKU patients improved neuropsychological performance, particularly in tests of abstract reasoning and cognitive functioning. PHE concentrations in CSF were reduced after VIL treatment though serum PHE was not affected, suggesting that these amino acids may successfully compete with PHE for receptors on a common transport system and thus reduce entry of PHE into brain.

A multicenter, double-blind trial of effects of VIL counter-treament in adolescents and young adults has been completed. Fifteen subjects, both on and off diet, received either VIL or a control mixture of amino acids (CS) in a random sequence for four periods of 3 months each. Tests were administered before and at the end of each period.

During VIL periods, time to completion of the Attention Diagnostic Method were faster, showing shorter latency; reaction time for the Continuous Performance Test approached significance, and significantly fewer errors occurred on a sensory-perceptual task (finger tip writing).

Serum PHE concentrations were similar throughout the study. No adverse effects were noted. These data support our hypothesis that attention based performance during VIL periods was superior to either baseline or CS periods, and that VIL promotes improved central nervous system functioning even in the presence of high concentrations of PHE.

442.3 THE MESENCEPHALIC DOPAMINE-CONTAINING NEURONS IN THE WEAVER MOUSE. S. Roffler-Tarlov, A.M. Graybiel, B. Martin , and J. Kauer, Neuroscience Prog., Tufts Univ. Sch. Med., Boston, MA 02111 & Dept. of Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139

Restricted subdivisions of the mesostriatal projection contain severely reduced dopamine (DA) levels in striatum of the mutant mouse weaver. The anatomical pattern of loss is along functional lines so that the ventral striatum is much more affected than the dorsal striatum. The DA deficiency occurs postnatally and is not fully expressed until 1 month. DA content and the typical islandic distribution of tyrosine hydroxylase (TH)-immunoreactive fibers in the striatum appear normal at postnatal (P) day 7. We and others have observed partial depopulation of the weaver SNpc (cell group A9) but neither the magnitude nor the time of appearance of this deficit has been determined. We report here the first part of a quantitative study aimed at identifying which subpopulations of DA neurons in the midbrain are vulnerable and when during development the cellular deficit appears.

Transverse 20 or 30 um sections through the midbrains of a weaver homozygote and littermate control at each of 3 ages (P7, P21, adult) were stained for TH-like immunoreactivity. The positions of all TH-positive neurons (TH-ns) in a spaced series of sections were plotted with the aid of a computer imaging system. A histogram of the medial-to-lateral distribution of the TH-ns per 100 um was generated for each section from the caudal limit of cell group A8 to the rostral limits of cell groups A9 and A10. Total numbers of TH-ns per midbrain were estimated by integrating the areas under composite distribution curves for the entire midbrain.

The quantitative measurements suggest the following conclusions: (1) At adulthood there is a deficit in absolute numbers of TH-ns in the weaver midbrain (weaver 65% control). (2) Most of the loss of TH-ns occurs post-natally (at P7, weaver 94% control). (3) The loss is strongly expressed at P21 (weaver 80% control). (4) The loss appears spatially restricted (distribution curves for weaver and controls at P21 and maturity differ most in the middle part of the transverse dimension). (5) Since the islandic system disappears in the weaver after P21, the loss of cells occuring between P21 and maturity must include the cells giving rise to the dopamine island system.

The finding that most mesencephalic DA neurons are in place in the newborn weaver suggests that the deficit is not primarily one of initial migration of these neurons, as might be predicted from the cerebellar defect in weaver. However, we have also found that at P7, the capacity of striatal synaptosomes to accumulate [^{3}H]DA is already reduced by 50% in weaver. Thus before significant cell loss is detectable in total midbrain TH-ns, the weaver disease is detectable biochemically. Supported by NS20181 and MH00655.

442.4 EFFECT OF CONGENITAL INFECTION OF SHEEP WITH BORDER DISEASE VIRUS ON MYELIN PROTEINS. J.R. Moller*, G.A. Elder*, B.J. Potts* and R.H. Quarles* (SPON: S.L. Sabol). NINCDS, NIH, Bethesda, MD 20892.

Border disease (BD) of sheep is a teratogenic disorder caused by a virus in the genus Pestivirus (Togaviridae). When acquired congenitally, the virus causes decreased myelination throughout the CNS with an accumulation of abnormal glial cells in white matter regions. Inflammation and neuronal destruction do not occur.

Pregnant ewes were inoculated with BD virus at 50 days gestation and myelin proteins were quantitated in several regions of the CNS during prenatal and postnatal development of infected lambs for comparison to age matched controls. Myelin basic protein (MBP), proteolipid protein (PLP), and myelin-associated glycoprotein (MAG) were measured by densitometric scanning of Western blots and/or radioimmunoassay. The specific activity of 2'3'-cyclic nucleotide-3'-phosphodiesterase (CNPase) was measured enzymatically.

Deficiencies in the myelin proteins were detected as early as 116 days of gestation. At all ages examined, the deficiencies of myelin proteins were most pronounced in the cerebellum. In six month old infected lambs, MBP and PLP of the cerebellum were between 40 and 50% of control levels, whereas MAG and CNPase were between 70 and 80% of control. Similar results were obtained for the corpus callosum and spinal cord of infected lambs, but the deficiencies of myelin proteins were not as great. A common finding in all regions examined was that MBP and PLP were reduced more than MAG or CNPase.

These results are probably explained by a greater deficit of compact myelin in which MBP and PLP are localized, than of associated oligodendroglial membranes in which MAG and CNPase are concentrated. Similar results have been obtained in several dysmyelinating mutants pointing to common factors in virally and genetically caused hypomyelination.

442.5 **DORSAL ROOT GANGLION NEURONS FROM NORMAL AND TRISOMY 21 HUMAN FETAL TISSUE SHOW TETRODOTOXIN-SENSITIVE AND TETRODOTOXIN-INSENSITIVE SODIUM CURRENTS.** **P. Caviedes and S.I. Rapoport(SPON: C. Edwards). NIH, NIA. Lab. of Neuroscience. Bethesda, MD. 20892.**

Trisomy 21(Down's Syndrome) is expressed as abnormalities in electrical membrane properties of cultured human dorsal root ganglion(DRG) neurons from fetal tissue(Scott B.S. et al.,Develop. Bra. Res. 2, 257, 1982; Nieminen et al., Soc. Neurosci. Abs. 1986, Vol.12,1360). Trisomic neurons have decreased activation time constants of outward potassium currents, compared to control neurons.

In the present study, we examined the properties of sodium currents using the whole cell patch clamp technique. DRG neurons were obtained from abortuses 16 to 20 weeks of gestation. Cells were dissociated with trypsin, and cultured in collagen-coated tissue culture dishes for 5 to 40 days. Recordings were conducted at room temperature(24°C), and cells were bathed in a calcium-free medium(in mM: 35 NaCl, 100 tetraethylammonium chloride, 20 HEPES-Na, 5 KCl, 1 MgCl$_2$, 5.6 glucose. pH=7.2). In voltage clamp, 10 mV steps were given from a holding level of -70 mV. Conventional prepulse and two pulse protocols were used to study inactivation kinetics.

A slow sodium current was recorded in voltage clamp mode, in the presence of 4 uM tetrodotoxin (TTX). A fast, TTX-sensitive component was identified by subtracting the slow component from the whole current in absence of TTX. The difference between the integrated total currents showed that the slow component accounted for 90% of the total charge moving across the membrane. The results suggest that the slow component has an increased inactivation time constant (13.6 msec) in trisomic compared to control neurons (7.2 msec), measured at -30mV. These results indicate, as in fetal mouse DRG neurons, that inward current in fetal human DRG neurons is principally carried out by a slow TTXinsensitive sodium current. At present, we have no clear explanation for the slower inactivation observed in the slow current of the trisomic neurons, although it may contribute to the known lengthening of the hyperpolarizing afterpotential(Nieminen et al.). Experiments are presently being conducted in order to further elucidate activation and inactivation kinetics.

442.6 COMPLEX EFFECTS OF METHYSERGIDE, d-AMPHETAMINE AND FENFLURAMINE ON LEARNING IN AN ANIMAL MODEL OF ATTENTION DEFICIT DISORDER (ADD). J.T.Concannon and P.Damski*, Dept. of Pharmacology, Southeastern College of Osteopathic Medicine, North Miami Beach, FL 33162

Recently, it has been shown that d-amphetamine is able to reverse 6-hydroxydopamine-(6-OHDA)-induced T-maze deficits in developing rats, and that this effect was in turn reversed by pre-treatment with the dopamine (DA) receptor blocker haloperidol (Concannon and Damski, EPA, 1987), suggesting that amphetamine works by a DA mechanism. Others (Heffner and Seiden, Brain Res., 1982 244:81) have suggested that the effects of d-amphetamine in 6-OHDA-treated rats are due to serotonin (5-HT) mediation, since they were blocked by pretreatment with methysergide, a purported 5-HT antagonist,although learning was not measured in this study. Hence, methysergide may also modulate amphetamine's effects on T-maze learning.

In the attempt to test this notion, we utilized intracisternal injections of 6-OHDA (150 µg) or its vehicle in 5-day old rat pups after desmethylimipramine (DMI) pre-treatment and examined T-maze learning at 20-23 days of age. At 60 min prior to testing,animals received either no injection or methysergide at one of 3 doses (1.0, 4.0 or 8.0 mg/Kg). At 30 min prior to testing, animals received either saline, d-amphetamine (0.50 or 1.0 mg/Kg),or fenfluramine (9.0 mg/Kg). Hence, the experiment was a 4x4x2 factorial combination of methysergide dose (none, low, intermediate, high), drug treatment(saline, low amphetamine, high amphetamine,and fenfluramine), and brain status (6-OHDA vs. vehicle). Animals were then trained for 20 trials in a standard T-maze position discrimination task.

The 6-OHDA treatment produced a T-maze learning deficit that was reversed by d-amphetamine, although amphetamine was ineffective in normal rats. Fenfluramine, on the other hand, was ineffective in 6-OHDA-treated rats, but produced a (methysergide-reversible) learning deficit in normal rats. The lowest dose of methysergide blocked the effects of d-amphetamine in 6-OHDA rats, even though it reversed 6-OHDA-induced learning deficits when administered alone prior to saline. Higher doses of methysergide together with either d-amphetamine or fenfluramine improved learning in 6-OHDA rats, even though they were not beneficial when administered prior to saline. These results,in conjunction with ongoing activity studies, suggest that methysergide has mixed behavioral properties vs. amphetamine, acting as an antagonist at a low dose, and an "agonist" at higher doses. However, when effective, it only antagonizes the effects of fenfluramine. Most importantly, methysergide in low doses seems effective by itself in reversing T-maze learning deficits and perhaps should be screened in human ADD.

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442.7 SINGLE NA FLUCTUATIONS IN CULTURED NORMAL AND TRISOMY 16 DORSAL ROOT GANGLION (DRG) NEURONS FROM THE FETAL MOUSE. C.B. Orozco*, C.J. Epstein*and S.I. Rapoport. (SPONSOR: W. Alberts). Laboratory of Neurosciences. NIA. Bethesda MD 20892, and Depts. of Pediatrics and Biochemistry and Biophysics. University of California. San Francisco. CA 94143-0106.

Murine trisomy 16 is a model of trisomy 21 in humans (Down syndrome). DRG neurons in culture contain a fast, TTX-sensitive and a slow, TTX-resistant Na conductances. DRG neurons from T16 mouse embryos have a faster action potential, with higher rates of depolarization and repolarization and also higher fast and slow Na conductances than do normal neurons. In fetal DRG neurons the slow Na conductance is 3-4 times the fast, TTX-sensitive Na conductance. There is no available information about the properties of the slow Na conductance at the single channel level however. The purpose of this study was to characterize the single channel activity of the TTX-resistant component in trisomic and control neurons.

Single channel currents were recorded in cell-attach and in cell free (inside-out) modes, with a patch amplifier (EPC-7, List Electronic, FRG). DRG neurons were obtained and maintained in tissue culture as previously described (Orozco, et al. Dev. Brain Res. 32:111-122). Microelectrodes with 10 Mohm resistance were used. Gigaseals of 10 to 30 gohm were obtained. Voltage membrane was varied between +40 mV to -40 mV in the cell-attached experiments, and from +120 mV to +20 mV in the inside-out experiments. The pipette solution contained (in mM): NaCl 130, $MgCl_2$ 10, dextrose 25, sucrose 10 and Hepes-NaOH 10, at pH 7.4 with 10 uM TTX. The internal solution contained: NaF 28, Tris-HCl 90, $MgCl_2$ 2, TEA-Cl 5, $CaCl_2$ 1, EGTA-Tris 11 and Hepes-Tris 10, at pH 7.4. In some experiments 4-aminopyridine 7.5 mM was included.

In the cell-attached configuration, three different currents were recorded. Two spontaneous fluctuations carried inward currents, about 0.5 pA and 1.0 pA at -100 mV. An outward current also was observed when pulses were applied to the membrane, probably due to Cl. Both inward currents decreased as the membrane potential became less negative. The frequency of the larger component (8 pS) decreased in presence of 4-AP, suggesting movement of Na through a transient K conductance. The slope conductance of the smaller inward component was 2.7 pS in cell-attached configuration and 2.2 pS in the cell-free mode. The current inverted at +30 mV, close to Na equilibrium potential (V_{Na}= +39 mV), indicating high selectivity for Na.

These results suggest the presence of a small ionic channel associated with the slow Na conductance in DRG neurons. Additional experiments are required to see if Na also can move through an additional channel.

442.8 RETARDED FETAL GROWTH OF FOREBRAIN COMMISSURES IN BALB/c MICE: MODE OF INHERITANCE. D. Wahlsten and G. Smith*. Dept. of Psychology, Univ. of Waterloo, Waterloo, Ontario, Canada N2L 3G1

Although only a minority of adult BALB/c mice show a marked deficiency or absence of the corupus callosum (CC), in 17 to 18 day BALB fetuses almost all show retarded growth of both CC and the hippocampal commissure (HC). An index of retardation was derived from previous data on genetically normal mice (Wahlsten, J. Comp. Neurol., 1987, in press) using quadratic regression of the sum of CC and HC cross-sectional areas on fetal body weight. Because deviations from the curve were larger for older fetuses, the expected squared residual was also determined with quadratic regression. The index was then a standard score (z), the difference between actual CC+HC and the value expected from fetal body weight, divided by the expected residual. A z value less than -2.0 was indicative of significantly retarded commissure development.

The first study compared BALB/cWah, C57BL/6J, their F1 and F2 hybrids, and backcrosses of F1 to BALB. Percentages of n fetuses in the body weight range of 0.5 to 0.8g with z scores below -2.0 were as follows: BALB(100% of 14), C57 (0% of 13), F1(12% of 24), F1 X BALB(56% of 91), F1 X F1 (16% of 49). The frequencies of retarded CC+HC in F1 and F2 generations did not deviate significantly from Mendelian ratios for a single gene, but there was no evidence of bimodality in the frequency distributions. Furthermore, retardation in abnormal F2 hybrids was less severe than in BALB.

The second study examined fetuses of BALB/cByJ, C57BL/6ByJ, their F1 hybrid and seven recombinant inbred strains (D, E, G, H, I, J, K). All BALB fetuses had z scores less than -2.0, whereas all C57 and F1 hybrid fetuses were above -2.0. Among the recombinant inbreds, strain G was severely retarded to the same degree as BALB, and strain I closely resembled C57. Almost all fetuses of strains H and J were below z of -2.0, but they were not as retarded as BALB. Strains E and K showed mild retardation, in that their average z score was less than 0, but only about 50% of them were actually below z of -2.0.

The data from the crossing study and the study of recombinant inbreds do not support single gene inheritance of the retarded fetal growth of CC+HC in BALB. However, they are consistent with a two locus model, wherein one recessive gene produces major retardation and another with intermediate inheritance leads to milder deficits in commissure growth.

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442.9 **PREFRONTAL CORTICAL ATROPHY IN SCHIZOPHRENIA IS ASSOCIATED WITH SKULL ASYMMETRY** D.G. DANIEL*, M.S. MYSLOBODSKY, R. COPPOLA, & D.R. WEINBERGER Clinical Brain Disorders Branch, National Institute of Mental Health, St. Elizabeths Hospital, Washington, D.C. 20032

Recent observations that ventricular enlargement and cortical atrophy are detectable in young schizophrenic patients (Weinberger et al., 1982) are consistent with the possibility that structural brain changes antedate the disorder. It has been hypothesized but never proven that an organic brain injury early in life could predispose to schizophrenic morbidity. If the trauma occurred early in life it could conceivably affect skull architecture as well as damage the brain parenchyma. It was predicted that the magnitude of skull asymmetry might covary with that of other signs of brain damage. The occipital bone depth (OBD) and the occipito-median angle (OMA) were assessed bilaterally in the CT scans of 38 chronic schizophrenics (DSM-3) and an asymmetry index (AI) (R-L/R+L x 100) was computed. The OBD and OMA were measured blindly on the upper infraventricular slice and two low ventricular slices. Prefrontal cortical markings were analyzed on a zero to three scale (with half steps) on the cut through the foramina of Monroe. A positive correlation emerged (r=.52; p<.0005) between the occipital bone depth (OBD) asymmetry index and prefrontal cortical atrophy scores (FBA), meaning that increase asymmetry in the direction of a flatter left occipital bone predicted cortical atrophy. Moreover, a inverse correlation between the occipito-median angle (OMA) and the prefrontal cortical atrophy scores was obtained (r= -.38; p<.01). This correlation suggests that right occipital flattening unaccompanied by OMA asymmetry identifies a subgroup of patients with prefrontal cortical atrophy. These findings may be interpreted as supporting the hypothesis that in a subgroup of schizophrenics prefrontal cortical atrophy and abnormalities in skull architecture may result from a insult early in life.

442.10 QUANTITATION OF THE FAST SODIUM CHANNELS AS SAXITOXIN BINDING SITES IN CENTRAL NERVOUS SYSTEM OF NORMAL AND TRISOMY 16 FETAL MICE. J. Koistinaho*[1] C. Orozco*[1] F. Gusovsky[2], C.J. Epstein[3] and S.I. Rapoport[1]. [1]LNS,NIA,NIH, [2]NIDDK,NIH, Bethesda, MD 20892 and [3]Dept. of Pediatrics, Biochemistry and Biophysiology, Univ. of California, San Fransisco, CA 94143.

Murine trisomy 16 is an animal model of trisomy 21 in humans (Down syndrome). Previous electrophysiological studies have revealed a shorter duration of action potential and higher excitability in trisomy 16 dorsal root ganglion and spinal cord neurons after 2-3 weeks in culture, than in control neurons (Orozco et al. Dev. Brain Res. 32,111-122, 1987). Similar alterations have been observed in dorsal root ganglion neurons with trisomy 21. In addition, fetal mice neurons have been shown to possess fast, tetrodotoxin (TTX)-sensitive and slow, TTX-insensitive sodium conductances. Both sodium conductances are significantly higher in trisomy 16 compared to normal fetal mice. The difference may be due to higher conductances per channel or, to higher densities of sodium channels in the membranes of trisomic neurons compared to controls. To test the second hypothesis, we used [^{3}H]-saxitoxin (STX) binding assay to quantitate fast sodium channels in brains of fetal trisomic and normal mice.

Trisomic and euploid (control) mice embryos (13-14 days of gestational age) were obtained as previously described (Orozco et al. op. cit.). Brains and spinal cords were dissected out and homogenized in Tris-buffer. The suspensions were centrifuged at 1000g for 10 min. Supernatants were recentrifuged at 20,000g for 20 min. The pellet of membranes was reconstituted in Na-free buffer. Suspensions were incubated at 37^{o}C in Na-free buffer containing 2 nM [^{3}H]-STX at different concentrations of cold STX. Non-specific binding was determined in the presence of 10 uM TTX in the assay mixture. After 30 min the reaction was stopped by filtering the samples through prewetted glass fiber filters (GF/C) and rinsing the filters three times with cold Tris-buffer. Bound radioactivity was measured in liquid scintillation counter. Protein concentration was determined by BCA assay method.

At the 13-14 day of development, the number of binding sites was small in both groups. Trisomic neurons showed lower binding at all concentrations of cold STX. The maximal binding capacity was about 14% less in trisomic than in control neurons. The dissociation constant of [^{3}H]-STX-receptor complex was 2nM both in trisomic and control neurons. These findings indicate some arrest in development of fast Na channels in the trisomic embryos as compared with controls. It is likely that longer time of neuronal development is required in animals for expression of higher Na conductance.

442.11 VISUAL EVOKED POTENTIAL TO FLASH IN NORMAL AND MAM RATS. M. Donadio,* M. Lee, A. Rabe, G. Heaney,* A. Heaney,* & E. Sersen.* N.Y.S. Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y.10314

Gross anatomical differences are apparent between brains of normal adult rats and those whose mothers received an injection of methylazoxymethanol acetate (MAM, 30mg/kg on gestation day 15). MAM rats have a severe micrencephaly with the posterior cortical area affected most. To discern if a functional difference could be mirrored in this structural deficit, the visual evoked potential (VEP) to a flash was compared for normal versus MAM rats.

Four normal and 4 MAM rats were chronically implanted with 10-12 fine stainless steel wire cortical electrodes and 2 screw electrodes as a reference and ground (sinus and cerebellum respectively). Each VEP was the average of 100 flashes (.9 or 2.7 flashes/sec) given to a restrained rat; an epoch was either 280 or 440 msec. At least two sets of VEPs at 6 monopolar derivations were thus obtained at each session. The data discussed represent at least 3 such sessions per animal over a minimum period of 2 weeks. The two sites chosen for analysis generated the highest amplitude VEPs and were located at comparable points over the posterior cortex of normal and MAM rats.

Visual inspection of the VEPs showed good within-subject and within-group consistency, revealing a VEP pattern differentiating normal and MAM rats. Both normal and MAM rats contained a prominent peak around 40msec ($\bar{X}$=43.2 vs 43.0), often a lesser peak around 75msec ($\bar{X}$=73.8 vs 79.0), and then a rising positivity which usually reached maximum around 170msec ($\bar{X}$=162.4 vs 180.2); this positivity had variable multipeaks. The most salient difference was the presence of a high amplitude peak around 210msec in the normal rats, which rode upon, or followed, the preceding period of positivity; it was often enhanced at the 2.7 flash rate. In no instance was an MAM VEP seen to have this peak, although P170 was sometimes enhanced by the higher flash rate. Furthermore, overall amplitudes of the VEPs in normals were always higher than any of the MAM ($\bar{X}$=95.7 vs 37.8uV) with the highest peak always occurring later than 100msec. In MAM rats the early component (P40) was often the largest. Between 300 and 400msec, at a flash rate of .9, prominent components mimicking the potentials seen between 100 and 200msec, but at a lower amplitude, occurred in the normals. These components were not apparent in MAM VEPs.

In conclusion, the structural abnormalities of the MAM cortex mirror themselves in functional changes seen through their VEPs. Visual discrimination deficits of MAM rats may be related to the severity of loss of some of these components.

422.12 CEREBROCORTICAL MICRODYSGENESIS IN NORMAL HUMAN BRAINS. W.E. Kaufmann* and A.M. Galaburda, (SPON: S.Weintraub). Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

We have described in four consecutively studied brains of male individuals with developmental dyslexia the presence of cerebrocortical microdysgenesis (layer I ectopias and associated dysplasias) involving perisylvian cortices predominantly in the left hemisphere (Galaburda *et al., Ann. Neurol.* 18:222, 1985). In this form of microdysgenesis the majority of neurons are appropriately placed in the cortical plate. The arrangement of neurons in the superficial layers, however, is anomalous; neurons may be found in the molecular layer and, at times, even in the subarachnoid space; there is often distortion of the cortical curvature to form microgyria or brain warts. The anomalies in the dyslexic brains number between twenty and forty, detected after each brain has been embedded whole in celloidin, sectioned at 35μm, stained with cresyl violet, and analyzed every forty sections. The literature on these types of developmental anomalies shows that they can be found in normal brains studied at autopsy; the figures document involvement in five to thirty percent of cases, with the larger figure referring to individuals of dubious normality (Veith and Schwindt, *Fortschr. Neurol. Psychiat.* 44:1, 1976). Most of these reports state that the anomalies are few in numbers and predominate in the right inferior frontal region. However, none of these studies examined brains at as great a level of detail as we do our dyslexic brains. Therefore, we carried out a study of ten normal brains from the Yakovlev Collection at the Armed Forces Institute of Pathology that have been prepared and examined in a manner identical to that of our dyslexic sample. The normality of the subjects was ascertained from available clinical records accompanying the specimens. The ages ranged from 3.5 to 87 years; there were nine males and one female. Four of these brains showed abnormalities similar in type to those of the dyslexic brains, but in far smaller numbers and in different locations: two of the brains showed one right frontal ectopia each; a third brain showed one left temporal ectopia; the fourth brain showed one left frontal ectopia. Thus, our findings do not differ substantially from those previously reported: thus, as many as 4 out of 10 normative cases showed the present form of microdysgenesis. However, unlike the finding in the dyslexic brains, the anomalies were seen in small numbers (basically isolated ectopias), and there is no particular side predilection. (Supported by NIH grant HD 19819).

442.13 CONNECTIONAL ANOMALY IN ASSOCIATION WITH CEREBRAL MICROGYRIA IN THE RAT. A.M. Galaburda, G.D. Rosen, and G.F. Sherman. Neuroanatomical Dyslexia Lab, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

We have reported cerebral microdysgenesis (layer I ectopias and associated dysplasias), including at times microgyria, to be a consistent finding in the brains of male individuals with developmental dyslexia, and have suggested that these anomalies are accompanied by abnormal neuronal connectivity (Galaburda *et al., Ann. Neurol.* 18:222, 1985). In this type of microdysgenesis the majority of neurons are appropriately placed in the cortical plate. The arrangement of neurons in the superficial layers, however, is anomalous; neurons may be found in the molecular layer and, at times, even in the subarachnoid space; there is often distortion of the cortical curvature to form microgyria or brain warts. The disorder, in the human, is thought to reflect a noxious event taking place at five to six months of gestation. Similar anomalies can be induced in rodents with damage occurring near the end of the period of neuronal migration to the cortical plate.

During the course of a callosal section experiment in adult Wistar rats (see Rosen *et al., Soc. Neurosci. Abstr.*, this issue), we found that one of the animals that had had a callosotomy also showed the unusual finding of spontaneous microgyria involving the posterior-lateral neocortex of the left side. The brain had been cut in coronal sections and processed according to the Fink-Heimer method, and adjacent sections stained with cresyl violet for Nissl substance. The cytoarchitectonic anomaly consisted of a fused molecular layer containing nests of ectopic neurons, a poorly differentiated second layer continuous with layers II, III, and IV of surrounding cortex, and compressed layers V and VI consistent with curvature artifact. The antero-posterior extent of the anomaly was 900 μm. Examination of adjacent sections for terminal degeneration revealed abnormal connectivity: there were unlaminated, dense projections from the pial surface to the depth of layer II-IV; a projection-free zone corresponding to layer V surrounded this zone of projection, with occasional projection-rich bridges to a layer VI that also contained dense projections. This is in contrast to the usual pattern of callosal terminations in this region, which consists of highly laminated projections to layer I, III, and IV (skipping layer II and the subpial portion of layer I), a layer V devoid of projections, and a projection-rich layer VI. This is also in contrast to the finding in reeler mouse, which demonstrates no alterations in connectivity to its inside-out cortex, an anomaly thought to reflect an earlier dysgenetic event. Anomalous projections in the present case may reflect preservation of the primitive, undifferentiated pattern or of targetting errors, as well as abnormal sprouting during a period of great plasticity. (Supported by NIH grant HD 20806).

442.14 NEUROPEPTIDE ARCHITECTONICS IN THE BRAIN OF THE NEW ZEALAND BLACK MOUSE. G.F. Sherman, J. Stone*, G.D. Rosen, and A.M. Galaburda. Neuroanatomical Dyslexia Lab, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

New Zealand Black (NZB) mice spontaneously express autoimmune disorders (Howie and Helyer, *Adv. Immunol.*, 9: 215, 1968), developmental cerebrocortical anomalies (Sherman et al., *PNAS*, 82: 8072, 1985), and problems with learning active avoidance tasks (Nandy et al., *Life Sci.*, 33: 1499, 1983). The emerging picture is comparable to that of male dyslexics, who show similar developmental brain anomalies (Galaburda et al., *Ann. Neurol.*, 18: 222, 1985), have learning problems particularly involving language, and, together with their families, may show increased rates of certain autoimmune and allergic disorders (Geschwind and Behan, *PNAS*, 79: 5097, 1982). The brain anomalies in both groups consist of nests of neurons in layer I of the neocortex, predominantly on one side of the brain, with distortion of adjacent cortical layers. It is believed that these types of anomalies have their origin during the last stages of neuronal migration or shortly thereafter. Our recent work has been aimed at establishing the NZB mouse as an experimental model for the study of the developmental pathology seen in dyslexia.

Reduction in the volume of the neocortex and caudate-putamen complex, and in the number of cholinergic neurons in the basal forebrain region also has been reported in the NZB brain (Zilles, *Senile Dementia of the Alzheimer Type*, 1985). In order to assess whether cytoarchitectonic anomalies are expressed at the level of chemical markers of functional specialization, we employed immunohistochemical techniques to identify vasoactive intestinal peptide (VIP), cholecystokinin octapeptide (CCK-8), and somatostatin in the cerebral cortex of NZB and control mice. Five adult NZB mice and five DBA control mice were perfused with 0.9% saline followed by 2% paraformaldehyde/0.1% glutaraldehyde, and frozen sections were cut at 30 μm. For each neuropeptide series every fifth section through the entire length of the cerebral cortex was stained using polyclonal antibodies (Immuno Nuclear Corporation) and counterstained with methyl green. An additional series from each brain was stained with cresyl violet to allow for the determination of cytoarchitectonic boundaries. A Gould FD-5000 image analyzer interfaced with a VAX 11/750 minicomputer is used for measurements and quantification of the histological material. Architectonic areas are parceled on the Nissl series, overlayed onto digitized immunohistochemistry images, and the architectonic borders transferred. The architectonic areal and laminar topography of each peptide and the number of positively stained cell bodies are determined. Comparisons will be presented for the experimental and control strains and for NZB mice with and without cytoarchitectonic anomalies. (Supported by NIH grants HD 19819 and 20806).

422.15 **INCREASED DOPAMINERGIC SENSITIVITY FOLLOWING PRENATAL MPTP AND MPP$^+$ TREATMENTS IN RATS.**
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Though MPTP produces a Parkinsonian syndrome in humans and monkeys, these effects are not observed in rats (Chiueh et al, Psychopharm., Bull. 20:548-553, 1984). However, MPTP has been reported to exert a selective destruction of embryonic rat dopamine (DA) neurons in culture (Mytilineou and Cohen, Science 225, 529, 1984). The present study examined whether prenatal administration of either MPTP or its active metabolite, MPP$^+$, would produce any neurotoxic effects in rats.

Timed-pregnant Sprague-Dawley rats were injected daily from E13 until birth with either 10 mg/kg MPTP or MPP$^+$ i.p., or saline vehicle. The offspring were tested for spontaneous nocturnal behavior and DA-stimulated behaviors using Digiscan-16 activity monitors (Omnitech Electronics) at various ages. For DA-stimulated behavior, animals were given either 1.0, 3.0, or 5.0 mg/kg amphetamine i.p. or 0.2 mg/kg apomorphine s.c. and their behavior was monitored for ninety minutes. Visual stereotypy ratings were correlated with various locomotor parameters.

Litter sizes in the three groups were similar, however, both drug-treated groups displayed significantly lower weights (through day 22) and delayed eye-opening and hair development. During spontaneous activity testing MPTP-treated pups were significantly hypoactive relative to controls at day 21 in ambulation and rearing parameters, while prenatal MPP$^+$ treatment produced hyperactivity in these two parameters at day 28. These transient differences were not seen at days 15 or 50. Across all time periods both MPTP and MPP$^+$ groups displayed increased sensitivity to apomorphine-induced stereotypy. MPP$^+$ and control groups displayed hyperactivity to 1.0 mg/kg amphetamine, but MPTP animals demonstrated significantly less rearing and ambulation at this dose. Both MPTP and MPP$^+$ groups displayed significantly greater stereotypy than did controls at higher doses of amphetamine. Dopamine, DOPAC and HVA levels were similar in all three groups at day 15.

These results demonstrate that prenatal administration of MPTP or MPP$^+$ in rats produces permanent increases in the sensitivity of striatal post-synaptic receptors. The amphetamine results and neurochemical findings indicate that these effects are not due to gross neurotoxic actions of MPTP on fetal dopaminergic neurons. Further studies need to elucidate the mechanism(s) underlying the long-term receptor sensitivity changes seen in rats prenatally exposed to MPTP. Supported by University Research Council, Psychiatry Dept. Intramural Grant and Omnitech Electronics, Inc.

422.16 USE OF A VISUAL PROSTHESIS AND NEOCORTICAL DEVELOPMENT IN INFANT MACAQUES. B.J. Sonnier, D.H. Warren*, A.H. Riesen, and E.R. Strelow* Dept. of Psychology, Univ. of California, Riverside, CA 92521

We are examining infant stumptail macaques (M. Arctoides) reared from birth to three months of age with electronic sonar sensory substitution devices to assess correlations between development of spatial-motor behavior and developmental changes in the neocortex. Groups of animals are reared as follows: 1) blind infants with the Trisensor Aid (TSA) in continuous use, blind control groups with 2) silent, dummy versions of the TSA or 3) sound-making but non-functional versions of the TSA, and 4) sighted, colony-reared infants. Cage observations and tests of spatial-motor behavior are made throughout the three month period, then animals are sacrificed and the neocortex is examined, using Golgi-Cox, microscope, and computer (Bioquant II) techniques. We have now sampled several neocortical areas from groups 2 and 3 to compare with samples that were reported for groups 1 and 4 at Neuroscience in 1985 and 1986.

Preliminary surveys have been completed in the following areas: M-I (precentral motor), M-II (supplementary motor), S-I (primary somatosensory), S-II (secondary somatosensory), Visual (areas 17, 18, and 19), Frontal, Posterior Parietal (inferior parietal lobule) and Auditory (areas 41 and 42). Our samples show some effects or changes in all laminae examined (II, III, IV, and V) and in both stellate and pyramidal neurons. Some effects are much more complex than changes in cell size or differences in dendritic numerical parameters. For instance, there appear to be changes in orientation and/or organization of some dendritic patterns. Some of these shifts in normalcy are slight, yet any noticeable change may be significant to the animal in which it occurs.

Our log indicates there was more device malfunction with the second infant than with the first one from group 1, and effects reported for the first animal are not as pronounced in the second one. Another problem concerns evaluation of the deprivation effect, which is always present to some degree in our single-housed, surrogate reared infants of this species. Rearing a sighted infant in one of the cage environments used for animals in groups 1, 2, and 3 would provide some means of assessing these effects.

It appears that continuous input of nonrelevant device signals or "noise" that fails to provide useful information about the spatial environment to the blind infant is disruptive to both neocortical and spatial-motor behavioral development. These animals do not appear to develop a consistent response system, or as some would say, do not develop a reliable neural network for appropriate responding over time. Apparently, intermittant "noise" produces some negative effects too. Learning to ignore either continuous or intermittant "noise" appears to interfere with learning to use spatially-relevant device generated information and other nonvisual cues, although deficits in hearing are not indicated by our tests.

442.17 RADIATION-INDUCED REDUCTION OF THE RAT DENTATE GYRUS. COMPENSATORY CELLULAR AND CONNECTIONAL CHANGES. J. Zimmer*, P.H. Poulsen*, Th.W. Blackstad* and M.J. West. Inst. of Anat. B, Univ. of Aarhus, Aarhus, Denmark and Inst. of Anat., Univ. of Oslo, Oslo, Norway.

X-irradiation of the hippocampal region of newborn rats selectively reduces the formation of dentate granule cells, which form after birth in contrast to other neurons in the region. In this study we have examined the effects of such X-irradiation on the dendritic differentiation of the residual granule cells and their afferent and efferent nerve connections. The hippocampal region on one side of the brain of newborn rats was X-irradiated (600 rad). After 3-10 months one set of brains were stained with the Timm method in order to visualize the dentate and hippocampal cell layers and the different terminal fields. The absolute volumes of these were estimated, and the irradiated and non-irradiated hippocampi of the individual rats compared. Other brains were Golgi-impregnated, and 3-D reconstructions of granule cells made by computer to investigate the size and shape of the dendritic tree.

With decreasing volume of the granule cell layer, reflecting a reduced number of cells, the volume of the afferent, perforant path (PP) zones in the molecular layer also decreased, but not to the same degree. This suggests that remaining granule cells respond to an increasing "surplus" of afferents by dendritic growth. Corresponding changes were observed for the ratio between the dentate mossy fiber projection (to hilus and CA3) and the granule cell layer, indicating compensatory axonal growth and/or nerve terminal formation.

The volume of the PP zones in CA3 was normal or larger than normal despite a reduction in the CA3 pyramidal cell layer. The expansion occurred both by formation of an aberrant projection to extended pyramidal cell basal dendrites (Laurberg and Hjorth-Simonsen, 1977) and by hyperdevelopment of the projection to the apical dendritic field.

In the Golgi-stained material the size and shape of the granule cell dendritic tree varied. One established fact was the development of anisotropy of many granule cell dendritic arbors in the X-irradiated fascia dentata. The longer axis was perpendicular to the dentate septotemporal axis.

The results demonstrate that reduction of a specific neuronal population can induce a) a compensatory increase in the neuropil layers containing the dendrites of the remaining neurons, - b) a corresponding relative increase in their axonal projection, - and c) a shift and expansion of afferent projections to an adjacent neuronal population. The analysis of individual, Golgi-impregnated cells further demonstrates the role of the afferent input in the shaping of the dendritic tree.

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442.18 **DEVELOPMENT OF WATER MAZE LEARNING IN THE NEONATAL TWITCHER MOUSE**
Ch.E.Olmstead, Jason Mnich* and Jennifer Simmons*. UCLA/MRRC Research Group, Lanterman Developmental Center, Pomona, CA 91769

The mutant twitcher mouse is an enzymatically authentic model of globoid cell leukodystrophy, a recessively transmitted deficiency of galactosylceramidase. Although clinical symptoms are not seen until well into the third week of life, we have reported retardation of both neurological and neurobehavioral indices of development prior to that time. The present study reports the development of swimming and spatial learning in the water maze in the twitcher mouse and its normal and heterozygous littermates beginning at 14 days of age.

The subjects were offspring of pairs of mice heterozygous for the twitcher mutation (C57Bl/6j/twi). The maze was in a large room with abundant visual cues, including lamps and windows, and was 53cm in diameter filled with water to a depth of 16cm with a 10x10cm platform at 12 o'clock. The mice were randomly placed at either 3, 6, or 9 o'clock and given 60s to find the platform. Five trials per day were run for either 5 consecutive or 3 alternate days, commencing at 14-19, 21, 24, 27, or 30 days of age. Five retention trials were run at 3,5,7,and 10 days following initial training. In addition to the latency and the distance traveled to find the platform, rating scales were used to assess other behaviors seen during the swimming.

Affected mice initiated at 14 days of age showed no clear learning over 5 consecutive days training, while their littermates showed significant within-session learning and some day-to-day transfer. The 15 day affected animals were somewhat better on the initial days, but the quality of the swimming deteriorated over the last two days of training and latencies increased accordingly. All normal and heterozygous animals older than 16 days of age at the outset of training learned the task by 19 days of age. Early normal animals (i.e., 14 days) showed clear within-session learning, but it was in the 17-18 day range that retention between days became apparent. Animals given initial training after day 18 showed excellent acquisition of the task in 1 to 2 five trial sessions whether on consecutive or alternate days. Retention of the task was excellent at all intervals from 1 to 10 days. Although some animals in all groups showed attempts to climb the walls, floating, and swimming aberrations on initial trials, these behaviors rapidly disappeared and there was a significant correlation between latency to find the platform and distance covered.

442.19 PURKINJE CELLS COUNTS IN HYPERSPINY PURKINJE CELL, A MUTANT WITH A CEREBELLAR DEFICIENCY IN 28K CALBINDIN. J. Mariani, F. Frédéric*, H. Shojaeian*, J.L. Guenet* and N. Delhaye-Bouchaud*. Institut des Neurosciences (UA CNRS 1199) and Lab. Neurophysiologie ontogénétique, Univ. P. et M. Curie, and Institut Pasteur, Paris, France.

Among the cholecalciferol induced calcium binding proteins (CaBPs) the 28K Calbindin is found mainly in the kidney and in the central nervous system, where the largest concentration is by far observed in the cerebellum. Histochemical studies using anti CaBP antibodies have clearly showned that cerebellar distribution of CaBP is restricted to the Purkinje cells (PCs), this being confirmed quantitatively by radioimmunoassay (RIA) of CaBP in the cerebellum of mutant mice lacking PCs (Parkes et al., Brain Res. 1985, 339 : 265-269). In a new mutant, hyperspiny Purkinje cells (hpc), the cerebellar content of CaBP measured by RIA showed a severe depletion (Mariani et al. Neuroscience abstracts, 1986,, 1231), although the PCs present in this mutant exhibited positive staining with an antiCaBP antibody.

The aim of the present study was to count the number of PCs in the cerebellum of this mutant to verify if the depletion in CaBP content was not due to a decrease in the size of the PCs population. Age matched hpc/hpc and control mice of the same strain (129SV) were perfused transcardially with Bouin-Hollande fixative, the brains were embedded in paraffin and cut serially at 10 µm in the sagittal plane; PCs were counted in every 15th section under the light microscope at a magnification of 1250. The number of PCs in each section was graphed as a function of the distance from the midline and the area under the curve was proportional to the number of PCs in the cerebellum. Because of the overestimation due to splitting of the nucleus during sectionning, the raw values of cell counts were corrected by the method of Hendry (Hendry, J. Neurocytol. 1976, 5 : 337-349). The deficit in PCs number in hpc/hpc mice as compared with control ones was only around 20% and cannot take into account the entireness of the depletion in the CaBP content revealed by the RIA. Thus, surviving PCs are likely to have a diminuted level of CaBP content. Since the PCs in hpc mutants exhibit morphological abnormalities (Guenet et al., J. Heredity 1983, 74 :105-108) especially of their dendrites, our counts strengthen the idea that this mutant could be a useful model to study the role of the 28K CaBP in the development and the function of PCs.

442.20 INFLUENCE OF BRIEF ETHANOL EXPOSURE DURING LATE GESTATION ON ACTIVITY OF ADULT C57Bl/6J MICE. R.M. Dumas*, R.K. Haddad and J.H. French*. (SPON: J. Shek). New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314.

Mice dams were gavaged on gestation day (GD) 15 with two equal doses of ethanol (total dose, 5.8 g/kg; time, 0 h. and 4 h.). Matched control dams were similarly treated with equivalent volumes of isocaloric dextrose solution and pair-fed for the subsequent 24 h. Live birth rates were the same in both groups. However, the neonatal (postnatal days 0-5) mortality rate was 5 times greater in the ethanol exposed group. This resulted in a somewhat reduced litter size. Both treatment groups were similar in gross appearance. Observations of home cage activity, circa weaning, noted that the ethanol exposed progeny were more easily aroused, initiated motor activities more rapidly when aroused, and perseverated them for longer periods of time.

Spontaneous locomotor activity of adult progeny was measured in an automated open field located in a dark room. One male and one female, selected from each litter in the two prenatal treatment conditions, were sequentially placed in the field and left unattended in darkness for thirty minutes during evening hours. Two 15-minute blocks of time, each comprised of three 5-minute trials, were recorded successively for each mouse. Preliminary results indicate that the ethanol exposed mice were no more active than their nutritional controls during the first time block (trials 1-3). The activity of the mice that were prenatally exposed to ethanol did not vary significantly across time. However, pair-fed control mice were consistently more active during the initial 15-minute time block than during the second. Chronic (Randall and Taylor, Teratology, 1979, 19: 305-312), as well as early, (Sulik, Johnston & Webb, Science, 1981, 214: 936-938), prenatal ethanol exposure are known to cause morphologic anomalies in the C57Bl/6 mouse. These anomalies include CNS malformations; persisting behavioral changes also have been reported, (Randall, Becker & Middaugh, Alcohol and Drug Research, 1985, 6: 351-360). Our current work indicates that a late and limited gestational exposure to ethanol also can be associated with behavioral changes in the C57Bl/6J strain.

TROPHIC AGENTS I

443.1 NEUROTROPHIC ACTIONS OF THERMAL (ARTIFICIAL) PROTEINS. F. Hefti, J. Hartikka, E. Junard*, P. Strang, A. Przybylski, G. Vaughan* and S.W. Fox. Departments of Neurology and Pharmacology, and Institute for Molecular and Cellular Evolution, University of Miami, Miami FL 33101.

Thermal proteins are formed when mixtures of amino acids are heated under simulated geological conditions. These polymers are evolutionary precursors of modern proteins, according to recent studies of amino acid sequence (Ivanov and Fortsch, Origins Life 17:35, 1986). Some of these thermal proteins assemble to cell-like structures with properties suggesting primitive neurons. These findings promoted us to test for effects of thermal proteins on modern neurons. We found that these compounds promote survival and fiber elongation of cultured rat brain neurons.

Cultures were prepared from dissociated cells of the forebrain of fetal rats (E16). The cells were plated at low density (50,000/sq.cm) in dishes coated with poly-lysine and grown in serum-free DMEM or L-15 medium. Cells grown in control medium degenerated within 1-3 days after plating. The addition of some of the thermal proteins resulted in a pronounced prolongation of the survival of cultured neurons. After 4 days, when control cultures had completely degenerated, treated cultures contained 63% of the originally plated cells. All surviving neurons had various long processes and, using specific neuronal markers, >95% of the surviving cells were identified as neurons. Treated cultures degenerated after app. 10 days after plating. Effective concentrations (EC50) of thermal proteins ranged from 3 to 30ug/ml. All active thermal proteins contained aspartic acid and a hydrophobic amino acid. A copolymer poly-(glu,ala,tyr, 6:3:1) synthesized from amino acid carboxyanhydrides (Sigma) produced similar effects as effective thermal proteins. The EC50 of this polymer was app. 0.3ug/ml. Effective thermal proteins acted in a highly specific manner, since, under the same experimental conditions, various control peptides (e.g., asp-phe), proteins (e.g., BSA) and growth factors (NGF, EGF, FGF, insulin) failed to promote the survival of neurons. The effect of thermal proteins was, at least in part, due to their binding to the culture substrate. The effect of thermal proteins was antagonized by heparin, but not by heparan sulfate or chondroitin sulfate.

443.2 NEUROTROPHIC ACTIVITY OF THE TRICYCLIC ANTIDEPRESSANT, CLOMIPRAMINE, ON SYMPATHETIC NEURONS IN CULTURE. T.T. Quach*, A.M. Duchemin*, B.K. Schrier+ and R.J. Wyatt. (SPON: M. Voigt). Neuropsychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. and +Laboratory of Development Neurobiology, NICHD, NIH, Bethesda, Maryland 20014

Tissue cultures of dissociated neurons have been used to detect neurotrophic factors and study their properties. They provide convenient assays for quantification of trophic activity (Manthorpe et al., Brain Res. 267:47-56, 1983). The survival of the neurons in response to an added factor can be measured and used as an index of the amount of trophic activity present.

Cultures of dissociated neurons from paravertebral sympathetic ganglia of 12-day-old chicken embryos were used in this study. These cells are very dependent upon the support of exogenous trophic factors for their survival in culture. Quantification of trophic activity was assessed either by direct count of surviving neurons under a phase contrast microscope after fixation and staining, or by measuring the incorporation of radioactive molecules added in the culture. A good correlation was found between direct cell cound and amount of incorporation of ^{35}S-methionine and ^{3}H-uridine. These two radiolabeled compounds were used for neurotrophic activity quantification. In each assay, neuronal survival obtained with an excess of Nerve Growth Factor (NGF) was used as maximal value. In negative control cultures, unsupported by exogenous factors, survival was less than 20% of the NGF values.

The addition of clomipramine, in the culture medium, resulted in an increased number of surviving neurons; 80% of the NGF effect. The clomipramine-induced neuron survival was concentration dependent and occurred with an apparent Kact of 0.1 µM. Maximal effect occurred at 0.3 uM. Incorporation of ^{35}S-methionine and ^{3}H-uridine in the cell cultures were also increased by the addition of clomipramine, with a similar Kact of 0.1 µM.

Sympathetic neurons require exogenous NGF or other neurotrophic factors for their survival in culture. Sympathetic neurons also have been found able to survive in the absence of NGF, with the tricyclic antidepressant, clomipramine.

443.3 THE EFFECT OF POLYAMINES ON MITOCHONDRIAL CA^{++} TRANSPORT IN ADULT AND DEVELOPING RAT BRAIN. J. R. Jensen, G. Lynch and M. Baudry. Center for the Neurobiology of Learning and Memory and Dept. of Pyschobiology, Univ. Calif. - Irvine, CA 92717.

The polyamines, spermine and spermidine, have been shown to increase the rate of Ca^{2+} transport by mitochondria from several tissues including brain. As a consequence of the increased rate of transport, mitochondria buffer the extramitochondrial Ca^{2+} concentration ($[Ca^{2+}]_e$) at values close to the resting intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). In the present study we investigated further the characteristics and mechanism of the polyamine effect. In addition, since polyamine levels are high in early postnatal brain, we compared the effect of spermine on mitochondria isolated from adult and neonatal rat brain.

Spermine not only lowered the steady-state $[Ca^{2+}]_e$ of isolated mitochondria as previously reported, but also dramatically compressed the rise in $[Ca^{2+}]_e$ normally seen with the loading of up to a total of 20 nmol Ca^{2+}/mg prot. Analysis of Ca^{2+} uptake kinetics indicated that spermine increases the rate of Ca^{2+} accumulation by increasing the affinity of mitochondria for Ca^{2+} and by decreasing the cooperativity of uptake. These effects are consistent with an allosteric activation of the Ca^{2+} uniporter by polyamines. Moreover, the effects are more pronounced in mitochondria from developing brain. The stimulatory effect of spermine was maximal at about postnatal day 10 and steadily decreased afterwards to reach adult values by postnatal day 35.

A short-term allosteric activation of Ca^{2+} uptake by Ca^{2+} was also observed; when a large amount of Ca^{2+} was added in a single load, $[Ca^{2+}]_e$ would return to values markedly lower than the pre-addition baseline. This effect was amplified in mitochondria from developing brain and was entirely blocked by spermine, suggesting both that spermine can substitute for Ca^{2+} at a allosteric site of the Ca^{2+} uniporter and also that the enhancement of the spermine effect described above is due to the greater cooperative character of Ca^{2+} uptake by neonatal brain mitochondria.

Since recent evidence supports the view that mitochondria function as Ca^{2+} buffers during periods of increased $[Ca^{2+}]_i$, changes in brain polyamine levels could have important effects on the regulation of $[Ca^{2+}]_i$. In addition, the enhanced stimulation of mitochondrial Ca^{2+} transport by spermine observed during the early postnatal period, coinciding with higher polyamine concentrations, suggests that $[Ca^{2+}]_i$ might be regulated at a narrower range of concentrations in developing brain. Furthermore, since a variety of growth factors and hormones produce a rapid induction of ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis, our results suggest an additional possible link between these factors and levels of $[Ca^{2+}]_i$.

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443.4 SYNTHESIS AND LOCALIZATION OF INSULIN LIKE GROWTH FACTOR mRNAS IN RAT BRAIN. M.A. Hynes, P.J. Brooks*, J.J. Van Wyk*, A.J. D'Ercole*, and P.K. Lund*. Curriculum in Neurobiology and Depts. of Pediatrics and Physiology, Univ. of North Carolina, Chapel Hill, NC. 27514.

Somatomedins, or insulin like growth factors(IGFs) are peptide growth factors which have mitogenic and differentiating actions. Recently, IGF-I and IGF-II mRNAs were detected in fetal and adult rat brain (Lund et al. J. Biol. Chem. 261, 14539, 1987).The regional and cellular sites of synthesis of IGF-I and IGF-II in rat brain however, are not yet defined. In the present study we analysed IGF-I and IGF-II mRNAs in cortex, cerebellum, hippocampus, hypothalamus and brain stem of neonatal and adult rat brain by Northern blot hybridization and the distribution of IGF-I immunoreactivity (IGF-I-ir) in adult brain regions by RIA. The cellular sites of synthesis of IGF-II mRNAs in adult rat brain were investigated also by in situ hybridization histochemistry (ISHH).

Using a rat IGF-I cDNA probe, four IGF-I mRNAs of 7.5,4.7, 1.7 and 1.2 kb were detected in all regions of neonatal and adult brain. No major differences in IGF-I mRNA abundance were observed in neonatal versus adult brain or in the different brain regions. Consistent with this finding were observations of similar levels of IGF-I immunoreactivity (IGF-I- ir) in different regions of adult rat brain. The four IGF-I mRNAs were detected also in adult rat pituitary and levels of IGF-I-ir were 4 fold higher in adult rat pituitary than in any brain region. The detection of both IGF-I mRNA and IGF-I-ir in multiple brain regions and pituitary suggests that these are sites of IGF-I biosynthesis. Five species of IGF-II mRNA were previously identified in many fetal rat tissues including brain. Recent studies by other groups have shown that these multiple rat IGF-II mRNAs arise in part by use of two different promoters and different transcription initiation sites within the IGF-II gene. In the present study we used probes specific for each of these promotors to analyse IGF-II mRNAs in neonatal and adult rat brain. Both probes detected multiple IGF-II mRNAs in all regions of neonatal and adult rat brain examined. We found no evidence of region specific expression of one or other of the two IGF-II promotors in rat brain.

Cellular sites of synthesis of IGF-II mRNAs were analysed in adult rat brain by ISHH with synthetic oligomer probes complementary to trailer peptide coding sequences of rat IGF-II mRNA and to the two different promotors of the rat IGF-II gene. Positive hybridization to cells of the choroid plexus was observed with all three IGF-II oligomers and hybridization occurred throughout choroid plexus. A control oligomer, complementary to rat somatostatin mRNA, showed no hybridization to choroid plexus. These findings suggest choroid plexus as a primary site of IGF-II mRNA synthesis in adult rat brain. IGF-ir has been detected in cerebrospinal fluid by other groups and the present data suggest that this derives at least in part from IGF-II synthesis in the choroid plexus.

443.5 LOCALIZATION OF INSULIN-LIKE GROWTH FACTOR II IMMUNOREACTIVITY IN ASTROCYTES OF POSTNATAL RAT CEREBRUM. J.A. Paterson, S.David[#], S.Pylypas* and R.P.C. Shiu*. Depts. of Anatomy & Physiology, Univ. of Manitoba, Winnipeg, Man. R3E 0W3, and [#]Neurosciences Unit, Montreal Gen. Hospital Research Inst., Montreal,Quebec H3G 1A4.

The characteristics of the insulin-like growth factor II (IGF-II) peptides within the brain, as well as their cellular source, are unknown, although IGF-II mRNA transcripts are found in fetal and postnatal rat brain (Brown et al., J.Biol.Chem.261(28), 1986; Lund et al., Ibid. 261(31), 1986). Here we report the electrophoretic characteristics of the IGF-II peptides synthesized by brain tissue fragments in vitro, and we describe the presence of immunoreactivity for IGF-II in astrocytes in vivo and in vitro.

Fragments from 6-day old rat brains (cortex, hypothalamus, striatum) were incubated in the presence of ^{35}S-cysteine; after 18 hours the media were collected. The proteins immunoprecipitated by antiserum to rat IGF-II (gift of Dr.S.P. Nissley, N.I.H.) were analysed by polyacrylamide gel electrophoresis and fluorography. Two IGF-II peptides of molecular mass 8 and 10 kd were detected from brain regions studied. Also a larger peptide (33 kd) was detected, possibly the carrier protein complexed to IGF-II.

Aldehyde-fixed brains from 5-day, 24-day and adult rats were used for immunohistochemical studies. First, 24d old rat cortex was vibratome-sectioned and the sections were incubated overnight with the anti-IGF-II serum, or with non-immune serum as control. The sites of antibody localization were made visible by the PAP method. IGF-II positive cells showed immunoreactivity in both their cell bodies and in radiating processes; often positive processes were close to blood vessels. Ultrastructural study of the same tissue showed immunoreaction to be in astrocyte-like cells. For the fixed brains from 5d and adult rats, frozen sections were examined by double-label immunofluorescence using ahti-IGF-II serum and a monoclonal antibody to glial fibrillary acidic protein (GFAP). IGF-II immunoreactivity was found in cells that also contained GFAP, a confirmation of the astrocytic identity of these cells. In sections of the cerebrum from both 5d and adult rats, a majority of the IGF-II-immunoreactive cells contained GFAP.

Cerebral cortices of newborn rats were dissociated with trypsin and grown in Dulbecco's MEM with 10% fetal calf serum. The cultured cells were characterized by double-label immunofluorescence. Immunoreactivity for IGF-II was seen in many GFAP-positive astrocytes, both of the flat and the process-bearing types. A few IGF-II positive cells were GFAP-negative. In summary, our studies indicate that astrocytes are a major neural cell type containing IGF-II immunoreactivity; since brain tissue itself synthesizes IGF-II peptides, it is possible that astrocytes are an important source of this growth factor in brain.

443.6 EPIDERMAL GROWTH FACTOR RECEPTOR LOCALIZATION AND TRANSPORT WITHIN THE RAT BRAIN STRIATONIGRAL SYSTEM. F.M. Leslie, R.S. Broide*, K.P. Cavanaugh*, R.A. Bradshaw and J.H. Fallon. Depts. of Pharmacology, Biological Chemistry and Anatomy and Neurobiology, Univ. Calif. Irvine, Coll. Med., Irvine, CA 92717.

We have recently shown that epidermal growth factor (EGF) exerts neuronotrophic effects in neonatal rat striatum (Morrison, R.S., et al., Science, in press). In the present study, we have used in vivo autoradiographic techniques to examine the properties of EGF receptors within this region of adult rat brain. ^{125}I-labeled EGF (200,000 cpm in 300nl) was injected using stereotaxic coordinates into caudate-putamen. In the contralateral caudate-putamen, radiolabeled EGF was injected in a similar total volume with various concentrations (10-1000ng) of unlabeled EGF, or other unlabeled growth factors. Following various survival times, animals were sacrificed and their brains removed and rapidly frozen. Twenty micron sections were cryostat cut, mounted onto slides and dried on a hot-plate, prior to apposition to tritium-sensitive film. After an appropriate exposure period, the latent autoradiographic image was developed and the underlying tissue section stained with cresyl violet. Within any given brain region, the amount of bound radioactivity was quantitated by computer-assisted analysis of autoradiographic grain densities and comparison with a set of standards of known radioactivity. Our autoradiographic data indicate that radiolabeled EGF is retained at the site of injection for greater than 18 hrs. On the contralateral side, in which radiolabeled EGF was co-injected with unlabeled EGF, there was significantly lower retention of binding. Unlabeled EGF displaced radiolabeled EGF binding in a dose-related manner, with significant displacement still observed at the lowest (10ng) dose. Unlabeled TGF-α produced a displacement of binding equivalent to that of EGF, while insulin was inactive. Six to 18 hr after injection into the caudate-putamen, radioactivity was also apparent in substantia nigra, pars reticulata, but not on the contralateral side in which competing ligand was co-injected. Following an 18 hr survival, the radiolabeled material in both caudate-putamen and substantia nigra were found to be present in a TCA-precipitable fraction. These data are consistent with the hypothesis that EGF is bound to specific receptor sites within caudate-putamen, then internalized and transported in an anterograde direction within the striato-nigral pathway.

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443.7 BASIC FIBROBLAST GROWTH FACTOR AND EPIDERMAL GROWTH FACTOR EXERT DIFFERENTIAL TROPHIC EFFECTS ON CNS NEURONS. Richard S. Morrison, Robert F. Keating*, Veronica K. Culhane*, and Joseph R. Moskal (SPON: D. Spitzer) Albert Einstein College of Medicine, Bronx, New York 10467.

Basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) are potent polypeptide mitogens which have recently been shown to enhance the survival and extension of neurites from cortical and striatal neurons in primary culture. The present study was undertaken with the purpose of identifying other populations of neurons responsive to the trophic effects of these two mitogens. Primary neuronal cultures were established from parietal cortex or cerebellum using neonatal rat brain. Cells were plated at a density of 1.25×10^5 cells/cm^2 in DMEM/Ham's F-12 supplemented with 10% FCS, Pen/Strep and cytosine arabinoside. Eighteen hours later cultures were converted to a chemically defined medium (CDM) plus either bFGF or EGF. Cerebellar neurons exposed to EGF exhibited a 4-6 fold enhancement in the number of neurons surviving with processes greater than 100 um in length. Exposure to EGF significantly increased the number, length and arborization of processes. Survival of total neurons was also enhanced. However, in CDM alone or in the presence of CDM plus bFGF the number of surviving neurons declined with time in culture. In addition bFGF did not induce neurite extension above that seen with CDM alone. The concentrations of bFGF used in these experiments varied between 0.1 and 10 ng per ml. This was in marked contrast to EGF which stimulated neurite outgrowth at a concentration as low as 100 pg/ml. Half-maximal process outgrowth with EGF occurred at 290 pg/ml. In order to show that the bFGF used in these studies was active, the preparation was tested on cerebellar astrocytes. As little as 1 ng/ml of bFGF was effective in stimulating astrocyte proliferation. bFGF also stimulated survival and process outgrowth from cortical neurons as previously described (PNAS 83: 7537-7541, 1986). These results clearly demonstrate that different populations of neurons have varied requirements for trophic support.

443.8 SIGNAL TRANSDUCTION MECHANISMS THAT PROMOTE NEURITE OUTGROWTH IN CULTURES OF CNS NEURONS. Joseph R. Moskal and Richard S. Morrison. (SPON: A. Hirschfeld) Albert Einstein College of Medicine, Bronx, New York 10467

Basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) have recently been shown to enhance survival and neurite outgrowth from defined populations of neurons. The mechanism by which they promote survival and process outgrowth is not understood. Many hormones, neurotransmitters and growth factors are known to initiate their action after first binding to a cell-surface receptor. One consequence of this process is the transduction of receptor binding into an intracellular signal sufficient for eliciting the proper metabolic changes. Two transduction pathways in particular appear to mediate the action of many hormones and growth factors: 1) stimulation of protein kinase C (PKC) by diacylglycerol and Ca^{2+} and 2) stimulation of cAMP-dependent protein kinase (CAPK). Since compounds are available which stimulate both pathways at sites distal to receptor binding, we sought to determine if PKC and CAPK activation would mimic the trophic actions of bFGF and EGF. Addition of phorbol ester tumor promotors (80 nM), which directly stimulate PKC, enhanced survival and process outgrowth from cerebral cortical (Cx) and cerebellar (Cb) neurons. Phorbol-12-myristate-13-acetate (PMA), the most effective promotor, was also the most active in eliciting neurite outgrowth. Phorbol 12,13-dibutyrate (PDBu) was also effective. In contrast, 4-Beta phorbol, which is a weak promotor, had no effect on neuron survival or neurite outgrowth. The addition of dibutyrl-cAMP (dBcAMP)(1 mM) was effective only in promoting neurite outgrowth from Cb neurons. dBcAMP and PMA acted synergistically in promoting process outgrowth from Cb neurons. In contrast, Cx neurons responded only to PMA. In fact, PMA was almost as effective as bFGF and EGF in inducing neurite extension. Moreover, branching induced by PMA in Cx neurons was less marked than with the growth factors. Dibutyrl-cyclic GMP (1 mM) did not elicit any changes in survival or process outgrowth from either Cx or Cb neurons. Butyrate (2 mM), retinol (1 uM) and retinoic acid (1 uM) were also ineffective. These results demonstrate that 1) activation of PKC and cAMP-dependent protein kinase may be partly responsible for mediating the effects of trophic factors on CNS neurons and 2) the relative role played by each pathway may vary from one neuronal population to another.

443.9 TROPHIC EFFECTS OF FIBROBLAST GROWTH FACTOR ARE MEDIATED BY DIRECT INTERACTIONS WITH HIPPOCAMPAL NEURONS AND ASTROCYTES. Patricia Ann Walicke Dept. Neurosciences, UCSD, San Diego, CA 92138.

Both basic and acidic fibroblast growth factors (bFGF; aFGF) have been observed to have trophic effects on hippocampal neurons in vitro, increasing neuronal survival and process extension. In addition, both are mitogens for astrocytes. We have used two methods to demonstrate that the neuronotrophic effects of the FGF's are mediated by direct interactions with neurons. Highly purified neuronal cultures were prepared by growing hippocampal cells for 6 days in the presence of aphidicolin. Control cultures contained 0.1% astrocytes as defined by staining for GFAP. Cultures grown with aFGF contained 0.2% astrocytes, while those grown with bFGF contained 0.3-1.0% astrocytes depending on the concentration of bFGF. Under each condition, individual cultures which contained no detectable GFAP+ cells were obtained. Since neuronal survival was still increased by aFGF and bFGF in these glial-free cultures, it is unlikely that either exerts its neuronotrophic effects indirectly through astrocytes.

A second series of experiments examined the binding of bioactive ^{125}I-labeled bFGF to cultures of highly enriched hippocampal neurons or astrocytes by autoradiography. Label was found associated with neurons above a minimal concentration of 10,000 cpm/ml (about 100 pg/ml) and with astrocytes above 50,000 cpm/ml. The vast majority of cells labeled in either type of culture, and neurons and astrocytes could be distinguished by the different morphological patterns generated by the grains outlining the cells. The label fulfilled the normal criteria of specificity in that binding was blocked entirely by excess unlabeled bFGF, but not by insulin, transferrin, EGF or NGF. Diminished but detectable binding was present after treating the cells with heparinase, suggesting that bFGF was not simply binding to cellular glycosoaminoglycans. Excess cold aFGF partially inhibited binding of [^{125}I]bFGF to both neurons and astrocytes, suggesting that it too interacted directly with each cell type. If the cells were incubated at 37°C after binding [^{125}I]bFGF at 4°C, the pattern of label shifted from the cell periphery to the perinuclear region, consistent with the changes expected during internalization of the ligand. It appears that bFGF binds to and is internalized by both hippocampal neurons and astrocytes. The observation that bFGF or aFGF can increase neuronal survival in the absence of astrocytes and the evidence that bFGF binds to neurons in a manner suggesting the existence of a possible receptor together strengthen the hypothesis that the FGF's are true neuronotrophic factors.

443.10 **ACIDIC FIBROBLAST GROWTH FACTOR STIMULATES BOTH NEURITIC OUTGROWTH AND PROLIFERATION IN CULTURED ADRENAL CHROMAFFIN CELLS.** P. Claude•°, K.A. Gordon*•, I. Parada*•, P.A. D'Amore*† and J.A. Wagner*‡. •Wisconsin Regional Primate Research Center and °Neurosciences Training Program, University of Wisconsin, Madison, WI 53715; †The Children's Hospital and ‡Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

Polypeptide growth factors can have both mitogenic and differentiating effects on their target cells. Nerve growth factor (NGF) has both a neurite-promoting and a mitogenic effect on cultured adrenal chromaffin cells from 8-day old rats (Lillien and Claude *Nature 317:* 632, 1985). Acidic fibroblast growth factor (aFGF), a heparin-binding polypeptide purified from bovine retina, is not only a potent mitogen for endothelial cells (D'Amore and Klagsbrun, *J. Cell Biol. 99:* 1545, 1984), but also promotes the outgrowth of neurites from pheochromocytoma cells (Wagner and D'Amore, *J. Cell Biol. 103:* 1363, 1986). We undertook this study to determine how chromaffin cells would respond to aFGF and to compare the effects of the two growth factors.

Dissociated chromaffin cells were grown for 6 days in Medium 199 plus 20% charcoal-stripped fetal calf serum, and then scored for the proportion of cells bearing neurites or labeled on day 6 by a 24 hr exposure to ^{3}H-thymidine (^{3}H-thy). In the absence of added growth factor, less than 2% of the cells exhibited neurites or incorporated ^{3}H-thy. In the presence of saturating concentrations of 7S NGF (100 ng/ml) or aFGF (120 ng/ml in the presence of 100 μg/ml heparin), roughly 25% of the cells grew neurites and a similar number incorporated ^{3}H-thy. Heparin (10-100 μg/ml) substantially potentiated the neuritic response to aFGF but only modestly potentiated the mitotic response. Thus heparin, or a heparin-like molecule on the cell surface or in the extracellular matrix, may regulate the response of some neuronal cells to this soluble growth factor.

Although these responses to NGF and aFGF are similar in many respects, there are interesting differences. NGF is a long-term survival factor for these cells, while aFGF is not. The synthetic glucocorticoid dexamethasone markedly depresses both the neuritic and the mitotic responses to NGF, but depresses only the neuritic response to aFGF. In addition, while the neuritic effects of NGF and aFGF are additive, the mitotic effects are not. Thus, although both NGF and aFGF promote differentiation and proliferation in adrenal chromaffin cells, it is clear that they do not have identical effects, suggesting that they may influence different second messenger systems.

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443.11 **ACIDIC FIBROBLAST GROWTH FACTOR ENHANCES REGENERATION OF PROCESSES BY POSTNATAL MAMMALIAN RETINAL GANGLION CELLS IN CULTURE.** S.A. Lipton, J.A. Wagner*, R.D. Madison, and P.A. D'Amore*. Program in Neuroscience, The Children's Hospital & Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

Acidic and basic fibroblast growth factors represent distinct but homologous gene products. Previously, basic fibroblast growth factor had been shown to promote neuronal survival and process extension in primary cultures of fetal hippocampal and cortical neurons (Walicke et al., *PNAS* 83:3012, 1986; Morrison et al., *PNAS* 83:7537, 1986). In the present study we show that acidic fibroblast growth factor (aFGF) enhances the outgrowth of processes by a postnatal central neuron, the rat retinal ganglion cell (RGC).

RGCs were labeled by retrograde transport of the fluorescent dye granular blue which was injected into the superior colliculi of 4-10 day old rat pups; two days later the retinas were excised, dissociated, and cultured as previously described (Leifer, Lipton, Barnstable & Masland, *Science* 224:303, 1984). Process extension by RGCs was significantly increased (2.6 fold) in the presence of aFGF after 24 hr in culture, a time when aFGF did not enhance RGC survival. The addition of heparin (10 µg/ml) to the medium potentiated the action of aFGF on process length (3.5 fold increase), but heparin itself had no effect. In the presence of heparin, half-maximal process outgrowth occurred at an aFGF concentration of less than 40 pg/ml (2 pM). Since the centrally projecting processes of RGCs have already been formed in the living animal prior to dissociation, at least a portion of the process outgrowth in culture appears to represent a regenerative phenomenon. Statistical analysis of the increase in process growth revealed that aFGF with heparin contributed to both neurite initiation and elongation. The mean number of glial cells, identified with polyclonal antiserum against glial fibrillary acidic protein, was slightly increased in cultures receiving aFGF plus heparin, but this effect was variable, and these glial cells were not in contact with the solitary RGCs that were scored for regeneration of processes. Thus, glial cells probably did not exert a direct physical influence on the degree of process outgrowth observed in the solitary RGCs, although a humoral effect cannot be excluded. These results suggest that aFGF has a potent influence on the outgrowth of processes by a neuron in the mammalian central nervous system. The potentiation of this effect by heparin leads us to speculate that the interaction of aFGF with a heparin-like molecule located in the extracellular matrix (such as heparan sulfate proteoglycan) may produce similar effects *in vivo*.

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443.12 INCREASED LEVELS OF BASIC FIBROBLAST GROWTH FACTOR (bFGF) FOLLOWING FOCAL BRAIN INJURY. S.P. Finklestein, P.J. Apostolides*, P.A. D'Amore*, V.B. Domesick, and M. Klagsbrun*. (SPON: R.J. Baldessarini). Mailman Research Center, McLean Hospital, Belmont, MA; and Massachusetts General and Children's Hospital, Boston, MA.

Basic fibroblast growth factor (bFGF) is a heparin binding growth factor with potent angiogenic and neuronotrophic properties. We used an affinity-purified polyclonal antibody to an internal peptide sequence of bFGF to immunostain intact brain and injured brain in which a unilateral mechanical lesion had been made seven days previously. In the intact brain, bFGF immunostaining was found in cells of the pia and ependyma, as well as in round cell bodies scattered throughout the neuropil, presumably glia. In injured brain, marked increases of bFGF immunoreactivity was seen at the borders of lesions, localized densely to numerous cells resembling astroglia and/or macrophages.

Increased levels of bFGF was supported by studies of in vitro mitogenic activity on 3T3/BalbC cells. These cells respond to bFGF (as well as several other growth factors) by increased proliferation, as measured by ^{3}H-thymidine incorporation. In the intact hemispheres of control animals, 3T3-stimulatory activity was 49 ± 5 (N=9) and 13 ± 1 (N=7) units/mg protein in total homogenates and extracellular fluid preparations, respectively. In lesioned hemispheres, 3T3-stimulatory activity was increased to 106 ± 8 (N=8) and 28 ± 4 (N=6) units/mg protein ($p<0.001$ and <0.01 compared to control), respectively.

Local increases in bFGF concentrations may promote neovascularization as well as increased neuronal survival and sprouting following focal brain injury.

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443.13 SULFATED GLYCOSAMINOGLYCANS MODIFY GROWTH FACTOR-INDUCED NEURITE OUTGROWTH IN PC12 CELLS. Deborah H. Damon*, Patricia A. D'Amore* and John A. Wagner (SPON: V. Domesick) Div. of Ped. Onc., Dana Farber Cancer Inst. and Lab. of Surg. Res., The Children's Hosp., Harvard Med. Sch., Boston, MA 02115.

Glycosaminoglycans (GAGs), localized on the surface of cells and in the basement membrane, modulate the growth and differentiation of many cell types. Recent studies have shown that heparin, a GAG found in mast cells, potentiates the ability of acidic fibroblast growth factor (aFGF) to induce neurite outgrowth in pheochromocytoma (PC12) cells, a neuronally-derived tumor cell line used as a model system for the study of neuronal differentiation *in vitro* (Wagner, J.A. and D'Amore, P.A., J. Cell Biol. 103: 1363, 1986). We tested the hypothesis that other GAGS found on cell surfaces and in basement membranes were capable of modifying growth factor-induced neurite outgrowth in PC12 cells. Neurite-outgrowth responses (the percentage of cells bearing processes greater than 1/2-cell body in length) were assessed in the presence of growth factors and varying concentrations of GAGs 72 hours following addition of agents. We found that sulfated GAGs modified the biological activity of aFGF, basic fibroblast growth factor (bFGF) and nerve growth factor (NGF). The effects observed were complex and depended upon the specific GAG used, the concentration of the GAG and the growth factor.

Heparin dose-dependently potentiated aFGF-induced neurite outgrowth; the degree of potentiation increased with increasing heparin concentrations of 0.01 - 100 ug/ml. At concentrations greater than 100 ug/ml, heparin potentiation decreased. The maximally active concentration of heparin (100 ug/ml) increased the potency of aFGF 102-fold. Increasing concentrations of heparan sulfate (HS), dermatan sulfate (DS) and chondroitin sulfate (CS) correlated with increased activity of aFGF. The maximally active concentrations of HS (100 ug/ml), DS (10000 ug/ml) and CS (1000 ug/ml) increased the activity of aFGF 11-, 110- and 11- fold, respectively. Hyaluronic acid (HA) did not affect the neurite outgrowth-promoting activity of aFGF.

Heparin also dose-dependently altered the activity of bFGF. From 0.01 - 1.0 ug/ml, increasing concentrations of heparin correlated with increased potentiation. At concentrations greater than 1 ug/ml, heparin concentration was inversely correlated with potentiation. At concentrations greater than 10 ug/ml, CS dose-dependently increased the activity of bFGF on PC12 cells. Maximally active concentrations of heparin (1 ug/ml) and CS (1000 ug/ml) increased the potency of bFGF 7- and 5-fold. Concentrations of HS greater than 1 ug/ml were inversely correlated with bFGF-induced neurite outgrowth and 1000 ug/ml HS significantly inhibited bFGF activity. DS and HA (0.01 - 1000 ug/ml) had no effect on bFGF activity.

HS and CS (0.1 - 1000 ug/ml) dose-dependently potentiated NGF; maximally active concentrations of HS (100 ug/ml) and CS (1000 ug/ml) increased the potency of NGF 3-fold, while heparin, DS and HA had no effect. None of the GAGs had any effect on PC12 cell neurite outgrowth when added alone. The specificity of the activity of the GAGs was verified by selective degradation. Chondroitin ABC lyase eliminated the potentiating activity of DS and CS, but had no effect on heparin or HS activity. Conversely, heparinase and/or heparitinase had no effect on the activities of DS and CS. These data indicate that not only, heparin, but other sulfated GAGs found in the extracellular matrix, can alter the activity of neuronal growth factors *in vitro* and thus may also influence growth factor-mediated functions *in vivo*. Supported by NIH grants RO1-EY06454-02 (J.A. Wagner) and RO1-EY05985 (P.A. D'Amore).

443.14 BIOLOGICAL FUNCTION OF NEURON-PRODUCED PROTEOGLYCANS. K.E. Dow* and R. J. Riopelle. Queen's University, Depts. of Pediatrics and Medicine, Kingston, Ontario, Canada, K7L 3N6.

Proteoglycans are important constituents of the extracellular matrix and have been implicated in regulation of neurite outgrowth.

Glycosaminoglycans (GAGs), the carbohydrate chains linked to the protein core of proteoglycans, were extracted from $^{35}SO_4$-labeled spinal cord neuron or sensory neuron cells and neuron conditioned medium after 7 days in culture. After separation by cellulose acetate electrophoresis bands co-migrating with heparin (H), heparan sulphate (HS) and chondroitin sulphate (CS) standards were detected. Chondroitinase ABC (100 mU/ml) digestion of extracts resulted in a loss of radioactivity in the band co-migrating with the CS standard while heparitinase (300 mU/ml) digestion resulted in a loss of radioactivity of bands co-migrating with H and HS standards. This finding suggests heterogeneity within the class of heparan sulphate GAGs.

Co-incubation of neurons with β-D-xyloside (50 µg/ml) inhibited sensory neuron process formation on laminin to 65% of control after 21 hours in culture. Process formation at 4 hours in the presence of β-D-xyloside was no different than in control wells indicating that the β-D-xyloside was not directly toxic to the neurons. Media conditioned by high density neuron cultures for 7 days in the presence of β-D-xyloside had 30-40% less substrate-attached neurite promoting activity (SANPA) than media conditioned in its absence.

Heparitinase and chondroitinase digestions of conditioned medium were carried out and the medium assayed for SANPA with sensory neurons. Heparitinase digestion reduced SANPA of conditioned medium to 62% of control while chondroitinase digestion had no effect. Sensory neurons cultured on a laminin substrate in the presence of monoclonal antibody to heparan sulphate proteoglycan (HSPG) had significantly fewer processes than in untreated cultures.

These data suggest that embryonic neurons synthesize and release heparan sulphate and chondroitin sulphate proteoglycans. Proteoglycans of the heparan sulphate class have neurite promoting activity when bound to laminin. The heterogeneity of the neuron produced HSPGs and their ability to bind to defined constituents of the extracellular matrix begin to address molecular mechanisms that may subserve pathway guidance and specific connectivity during axonal growth and regeneration.

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443.15 LESIONING AND RECOVERY OF THE SEROTONINERGIC HIPPOCAMPAL AFFERENTS: DIFFERENTIAL EFFECTS OF GM_1 GANGLIOSIDE. G.Lombardi*,M.Beni*,V.Carlà* A.Consolazione* and F.Moroni. (SPON: L.Beani). Dept. of Pharmacology, University of Florence, 50134 Florence and FIDIA Res. Lab., Abano Terme (PD) 35031, Italy.

Gangliosides have been shown to reduce the damage after a mechanical lesion of the cholinergic or dopaminergic system (Wojcic M. et al.,Neuroscience,7:495,1982; Toffano G. et al., Brain Res.,261:163, 1983). However, the mechanisms of their action are still unknown, and they could either foster neuronal sprouting or prevent cell death in the vicinity of the lesioned area. In order to study ganglioside effects on the 5-hydroxytryptamine (5-HT) system we used two types of lesions of the 5-HT afferents to rat hippocampus. The first lesion consists in cutting unilaterally the fimbria-fornix using a razor blade (Moroni F. et al., Brain Res.,150:333,1978). Seven days after this procedure the 5-HT and the 5-hydroxyindolacetic acid (5-HIAA) content decreased by 78% and by 65% respectively in the dorsal and by 50% and 40% respectively in the ventral hippocampus. However, 60 days later a partial recovery, possibly due to a collateral sprouting, does occur. The second lesion consists in an electrolytic destruction of a mesencephalic area at the following coordinates: AP= 4.9, L= +1, H= 7.4 (Konig-Klippel 1963). This lesion decreases the 5-HT and the 5-HIAA content by 36% and by 20% respectively in the total hippocampus. A treatment with GM_1 ganglioside (30 mg/Kg/day for up to 60 days) failed to foster the sprouting of 5-HT neurons which was present in fimbria-fornix rats 60 days after the surgery. However, in rats having an electrolytic destruction of the mesencephalic area near the soma of scattered 5-HT cells innervating the hippocampus, a treatment with GM_1 ganglioside (30 mg/Kg/day/15 days) reduced the decrease of the hippocampal 5-HT.

The results of our studies indicate that GM_1 administration does not facilitate the sprouting of 5-HT neurons innervating the hippocampus when the fimbria-fornix pathways are severed, but it is able to reduce the damage of an electrolytic mesencephalic lesion possibly preventing the death of neurons in the vicinity of the lesioned area.

443.16 **ADMINISTRATION OF GM-1 GANGLIOSIDE RESTORES DOPAMINE CONTENT IN THE STRIATUM OF MPTP-TREATED MICE.** **M. Hadjiconstantinou and N.H. Neff, Department of Pharmacology, The Ohio State University College of Medicine, Columbus, Ohio USA 43210**

There is experimental evidence documenting that administration of GM-1 ganglioside stimulates the recovery of dopaminergic nigrostriatal neurons after different types of lesions. Apparently, GM-1 ganglioside administration has a regrowth-stimulating effect and/or a protective action against secondary retrograde degeneration following nerve terminals lesions. It may effect the activity of neurotrophic and neurite promoting factors and/or repression factors at the level of the lesion which influence regeneration. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induces parkinsonism in humans and monkeys and degeneration of nigrostriatal neurons in various species. We now report that the administration of GM-1 restores the dopamine (DA) content in the striatum of MPTP-treated mice. Mice were treated with MPTP, 30 ug/kg, i.p., for 7 days. This treatment resulted in a loss of neurons in substantia nigra and about a 60% decrease of DA and DOPAC in the striatum. On day 8, GM-1, 30 mg/kg, i.p., or the synthetic ganglioside analogue AGF-2, 20 ug/kg, i.p., was administered daily for 23 days. Drug treated control groups were evaluated as well. Treating mice with GM-1 or AGF-2 alone had no significant effect on the striatal content of DA or DOPAC. Both GM-1 and AGF-2 restored the DA content in striatum of MPTP-treated mice. This effect was dose- and time-dependent. Maximum effect was observed after about 3 weeks of treatment with 30 mg/kg, i.p., of GM-1. GM-1 increased the DOPAC content in the striatum of the MPTP-treated mice over control values, suggesting stimulation of DA synthesis. Indeed, when the DA synthesis was studied directly, we found that GM-1 administration caused a 2 to 3-fold increase above control DA turnover values in the striata of MPTP-treated mice.

443.17 INVOLVEMENT OF SUBCORTICAL CHOLINERGIC SYSTEMS FOLLOWING NEOCORTICAL LESIONS: EFFECT OF GANGLIOSIDES ON BIOCHEMICAL AND BEHAVIORAL CORRELATES. P.J.Elliott*, L. Garofalo* and A.C. Cuello. (SPON: B. Esplin).Department of Pharmacology, McGill University, Montreal, Canada, H3G 1Y6.

Alzheimer's disease includes pathological effects on cholinergic forebrain neurons. In an attempt to study the cholinergic aspect of this disabilitating disorder, animal models of the disease have been devised. These experiments generally involve lesioning of specific cell body or terminal areas of CNS cholinergic neurons. Such models produce the characteristic decrease in cholineacetyltransferase (ChAT) activity and cell loss as seen in the human condition. Whether this degeneration occurs retro- or anteroactive to the original insult is indispute. In the present set of experiments animals were unilaterally decorticated by disrupting the blood vessels which supply the cortex. This devascularizing lesion produces a retrograde-type degeneration of the cholinergic neurons originating from the nucleus basalis magnocellularis (NBM), as previously described (Sofroniew et al. Brain Res. 289:370-374, 1983; Stephens et al. J. Neurochem. 45:1021-1025, 1985). Forty-five subjects (male Wistar rats; 300-350g) were used in the study: 25 control and 20 lesioned subjects. 50% of each group were infused with the ganglioside GM1 (5mg/kg/day; 14 days) using minipumps (ALZET)connected to chronically implanted intracerebroventricular cannulae. One month following the initial lesion, various subcortical and cortical brain areas were assayed for ChAT activity. In agreement with previous data (Cuello et al Brain Res. 376:373-377, 1986) the cortical lesions produced a 30-50% decrease in ChAT activity in the ipsilateral NBM and this effect was prevented by application of GM1. Prior to the surgical manipulations and immediately before the subjects were sacrificed, each animal was tested in a variety of behavioral paradigms; sensorimotor testing, pain sensitivity, locomotor activity and memory testing (passive avoidance and Morris water maze). Correlations between the biochemical and behavioral data will be reported.

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443.18 GANGLIOSIDE-ENHANCED RECOVERY OF SPATIAL ALTERNATION PERFORMANCE AFTER UNILATERAL ENTORHINAL CORTEX LESIONS IN RATS. J.J. Ramirez*[1], B. Fass[2], D. Zengel*[1], P. Wright*[1], and S.E. Karpiak[3]. [1]Psychology Dept., Davidson College, Davidson, N.C.; [2]Anatomy Dept., Univ. Louisville Med. Sch., Louisville, KY; [3]Div. Neuroscience, NYSPI, Columbia Univ. Coll. Phys. Surg., N.Y., N.Y.

Exogenous gangliosides have been shown to improve behavioral recovery after brain damage. In our previous studies, rats treated with mixed gangliosides throughout 3 weeks of postoperative behavioral testing showed better retention of a learned alternation task than saline-treated rats after uni- or bilateral entorhinal lesions (Karpiak, 1983; Ramirez et al., 1987). The present study was performed to determine whether short-term ganglioside treatments would facilitate recovery of alternation behavior after unilateral entorhinal lesions. Gangliosides were administered during the early postlesion interval; i.e., the day preceding surgery and the next 6 days. This interval was just prior to the onset of crossed temporodendate sprouting, which has been implicated in the recovery of alternation (Loesche & Steward, 1977).

Male Sprague-Dawley rats sustained unilateral entorhinal lesions or craniotomies and were tested for retention of a preoperatively acquired alternation task (Y maze). Ganglioside- (50 mg/kg) and saline- (0.9%) i.m. injections were administered for 7 consecutive days beginning on the day before surgery. The rats were tested postoperatively until they attained criterion performance (3 consecutive days of 80% alternation), or for a maximum of 3 weeks.

Although the extent of damage was comparable between the two lesion groups, the ganglioside-treated rats performed better than the saline-treated rats. The former required fewer postlesion days to reach criterion performance.

Thus, a short-term regimen of ganglioside treatment is effective in promoting behavioral recovery after unilateral entorhinal lesions (c.f., Li et al., 1986). Since it has been suggested that exogenous gangliosides alter acute and long-term postlesion events (e.g., edema, membrane failure, sprouting), these events may contribute to the enhanced recovery observed in the present study. The relative degree to which these events contribute remains to be determined.

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443.19 GM-1 GANGLIOSIDES ARE INEFFECTIVE IN REDUCING AMPHETAMINE-INDUCED ROTATIONAL ASYMMETRY, BUT PROTECT RATS FROM APHAGIA AND ADIPSIA FOLLOWING COMPLETE NIGROSTRIATAL HEMITRANSECTIONS. G.L. Dunbar,+ P. Sapozhnikov,* and D.G. Stein. Brain Res. Lab., Clark University Worcester, MA 01610; +now at Dept. of Psychology, Central Michigan University, Mt. Pleasant, MI 48859.

Since the effect was first reported by Toffano et al.(1), studies have consistently shown that treatments with GM1 gangliosides attenuate the loss of striatal tyrosine hydroxylase (TH) levels and reduce rotational asymmetries in rats with partial hemitransections of the nigrostriatal pathway. However, subsequent work (2) shows that after extensive hemitransections of the nigrostriatal pathway, the effect of GM1 on striatal TH levels disappears. This study investigates whether extensive transections also eliminate the GM1 effect of reducing rotational asymmetry. Since these transections often cause aphagia and adipsia in rats, we also measured whether GM1 treatments could counteract the severe weight loss observed after this injury.

Seventeen, male albino rats were given complete hemitransections of the nigrostriatal pathway, following the parameters used by Toffano (2). One group (n=9) received daily IP injections (30mg/kg) of GM1 gangliosides (Fidia Research Laboratories), beginning immediately after surgery and continuing for two weeks. Another group (n=8) received the same regimen of IP injections of saline. Amphetamine induced ipsiversive rotations were assessed at postoperative days 2, 7, and 14. Daily body weights were taken for both groups.

Results revealed that gangliosides had no effect on the number of ipsiversive rotations during any of the testing days, but significantly reduced the amount of weight loss (6% vs. 13%). It is not known how gangliosides protect rats from aphagia and adipsia following this nigrostriatal transection but the protection observed suggests that it probably involves mechanisms other than dopaminergic innervation of the striatum by nigrostriatal fibers.

1. Toffano et al., 1983, Brain Res., 261: 160-170.
2. Toffano et al., 1984, J. Neurosci. Res., 12: 397-408.

+this study was supported by a grant from Fidia Research Laboratories Albano Terme, Italy.

443.20 GANGLIOSIDES GM1 AND AGF2 PROMOTE RECOVERY OF WORKING MEMORY FOLLOWING COLCHICINE-INDUCED GRANULE CELL LOSS IN RAT HIPPOCAMPUS. D. F. Emerich and T.J. Walsh. Dept. of Psychology, Rutgers University, New Brunswick, N.J

GM1 ganglioside promotes recovery of function following various forms of neural insult. This compound has been shown to attenuate the cognitive and motor impairments following colchicine (COL) induced granule cell loss in rat hippocampus (Emerich et al, Soc. Neurosci. Abs., 1985). These experiments further examined the ability of GM1 as well as its internal ester, AGF2, to attenuate the cognitive impairments resulting from the destruction of this population of neurons.

Rats were trained on a multiple component T-maze task (Chrobak et al, Br. Res., 1987). Animals were placed into one of two start/goal boxes located on either arm of the maze. They were trained to run down the stem of the maze and return to the alternate start/goal box for food reward (i.e., a non-match to sample task). The location of the start box was randomly alternated on each trial. The different aspects of this task may be characterized as a) a reference memory component which involves an invariant response of running down the stem of the maze and b) a working memory component in which the the animal must maintain some representation of the previous stimulus event (i.e., the start location) to perform accurately. Following acquisition of the task, rats were injected (IP) with either GM1 (30 mg/kg), AGF2 (10 mg/kg) or the saline vehicle (SAL) for three days prior to surgery and for 14 days thereafter. COL (3.5 ug site) or SAL was bilaterally injected into the dentate gyrus at two rostralcaudal locations. Both COL and SAL-treated animals exhibited a transient impairment on the reference memory component which recovered within 15 trials. GM1 and AGF2 did not alter the rate at which refernce memory recovered. While control animals required 55 trials to reach a criterion of 70% accuracy on the working memory component, rats injected with COL (COL-SAL) were markedly impaired on the working memory component and required 110 trials to reach the same level of performance. Rats treated with GM1 (COL-GM1) required 95 trials and rats treated with AGF2 needed 70 trials to reach this criterion. Thus, GM1 and, in particular, AGF2 enhanced the rate of recovery of normal working memory.

Histological analysis revealed that COL produced a 55% decrease in the thickness of both the superior and inferior blades of the dentate gyrus due to the marked loss of granule cells. This was accompanied by a slight decrease in hippocampal volume, mild ventricular dilitation, and a small (13%) decrease in the thickness of the CA1 pyramidal cell field. Neither GM1 or AGF2 decreased the extent of granule cell loss induced by COL. The beneficial effects of these neurotrophic agents are probably not related to a decrease in the impact of COL on its population of target neurons (granule cells) but rather could represent a reduction in secondary changes resulting from cell loss, or the facilitation of behavioral and/or neural reorganization that promotes functional recovery. These data further support the contention that certain gangliosides can facilitate recovery of function following destruction of specific populations of cells, a common finding in neurological diseases.

TROPHIC AGENTS II

444.1 VISUALIZATION OF NERVE GROWTH FACTOR BIOLOGICAL ACTIVITIES BY A CELL-BLOT TECHNIQUE USING GANGLIONIC NEURONS AS PROBES. B. Pettmann*, S. Varon and M. Manthorpe (SPON: C. Wiley). Dept. of Biology, Univ. California San Diego, La Jolla, CA 92093.

We have recently described a "cell-blot" technique which permits direct visualization of an active neuronotrophic factor after its resolution by SDS polyacrylamide gel electrophoresis and electrophoretic blotting to nitrocellulose. The nitrocellulose strip carrying the blotted trophic band is then soaked in antibiotics, blocked with ovalbumin, placed into specially designed troughs, extensively washed and used as a substratum for the culture of corresponding "probe" neurons in medium lacking the neuronotrophic factor itself. A vital dye is presented to the culture over the last two hours of incubation, to distinguish between living and dead neurons, and survival is quantitatively determined after fixation. A crucial implication of the success of this technique has been that neuronotrophic factors may exert their survival-promoting influence on their target neurons from a surface-anchored position, as well as from a humoral environment.

In previous work, this cell-blot technique was applied only to the Ciliary Neuronotrophic Factor (CNTF) from various chick and rat tissues, as probed by its standard target neurons from E8 chick ciliary ganglia. Both the technique and its biological implications would greatly gain if the same findings could be extended to i) other neuronotrophic factors and ii) other probe neurons. We report here that: (1) The 26 kd Nerve Growth Factor (NGF) dimer can also be successfully recognized with a cell-blot technique (and, thus, capable of operating after surface anchorage) -- provided certain procedural aspects are modified; (2) a variety of ganglionic neurons can be used to probe either NGF or CNTF, in precise correspondence with their known responsiveness in more traditional neuronal cultures; (3) the cell-blot systems permit the recognition of neuritic growth, as well as neuronal survival, responses; and (4) neuritic growth responses can differentiate between blotted NGF and CNTF effects on the same probe neurons.

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444.2 NEUROBLASTOMA CELL LINES AS A SOURCE FOR MULTIPLE NEURONOTROPHIC ACTIVITIES. D. Heymann*, J. Heymanns* and K. Unsicker. (SPON: European Neuroscience Association). Dept. of Anatomy and Cell Biology, D-3550 Marburg, F.R.G.

A variety of cell types, in particular glial and mesenchymal cells, are known to secrete neuronotrophic factors (NTFs) i.e. proteins that promote survival and differentiation of neurons both in vitro and vivo. There is now increasing evidence to suggest that neurons may also store and secrete NTFs (Unsicker and Lietzke, Soc. Neurosci. Abstr. 11, 313.6, 1986; Unsicker et al., Soc. Neurosci. Abstr. 1987). Since the availability of large quantities of well characterized homogeneous neuron populations is a prerequisite for a biochemical analysis of neuronal NTFs we have examined two neuroblastoma (NB) cell lines, the human IMR 32 and mouse C 1300 N2a, for putative NTFs. Extracts from both NB-lines promoted in vitro survival of embryonic chick ciliary (E8), dorsal root (E10, not E8) and lumbar sympathetic (E11) ganglia neurons. This spectrum of target cells is identical to that of the ciliary neuronotrophic factor (CNTF). CNTF has been isolated from embryonic chick eye (M_r = 20 500; pI = 5.0) and rat sciatic nerve (M_r = 24 000) and shown by us to bind specifically to heparin. Activities from NB cells were sensitive to heat (60°, 60 min) and chymotrypsin treatment and not blocked by anti-NGF(mouse)antibodies. Gel chromatography studies on IMR-extracts revealed an apparent M_r of 21 and 45 kD. In "cell blot" experiments (Carnow et al., J. Neurosci. 5, 1965-1971, 1985; i.e. bioassays on nitrocellulose paper onto which electrophoretically separated NB cell extracts had been transferred) ciliary ganglion neuron survival was exclusively seen on two distinct bands with apparent M_r of 24 and 48 kD, respectively. Extracts from C1300 cells, enriched on heparin sepharose, yielded 4 peaks corresponding to M_r 15, 21, 32 and 52 kD. In their ability to bind specifically to heparin the neuronotrophic activities from both cell lines show a common feature with CNTF and other growth factors. With regard to biological activities and molecular weight it therefore seems that IMR 32 and C1300 cell contain CNTF-like molecules. However, isoelectric focusing in IMR cell extracts yielded a single activity at pH 9 addressing ciliary ganglion neurons. These findings suggest that multiple NTFs are present in NB cells. Some of them may be related to CNTF and other heparin-binding growth factors.

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444.3 NEURONOTROPHIC FACTORS IN ADRENAL MEDULLARY CELLS: DEVELOPMENT, RELEASE AND MOLECULAR CHARACTERISTICS. K. Unsicker, D. Blottner*, D. Gehrke*, F. Stögbauer*, R. Westermann*, R. Fischer-Colbrie*, H. Winkler*, and P. Böhlen*. Depts. of Anatomy and Cell Biology, University of Marburg, F.R.G., Pharmacology, University of Innsbruck, Austria, and Biochemistry, University of Zürich, Switzerland.

Target-dependent neuronal death is a basic phenomenon in nervous system development. With regard to neuron-neuron interactions it would imply that neurons may store and secrete neuronotrophic factors (NTFs) that support survival and differentiation of their presynaptic counterparts. We have previously shown that proteins stored in chromaffin vesicles of bovine adrenal medullary cells promote in vitro survival of embryonic spinal cord, sensory and sympathetic neurons innervating the adrenal medulla in situ (Soc. Neurosci. Abstr. 11/2, p. 1083, 1985). We report here on development, release and further molecular characteristics of these NTFs. Development: High speed supernatants of homogenates from carefully dissected rat adrenal medullae (postnatal day (P) 2 through P 90) carried NTF activity for embryonic chick cilary (day 8; CG8), dorsal root (DRG8, DRG10) and lumbar sympathetic (SG11) ganglion neurons from P8 onwards, i.e. shortly after the onset of the functional preganglionic innervation of the medulla at P5 (ED_{50}: 10 µg protein/ml). Homogenates of adrenal cortex had weak activity for SG11, but none for the other neurons tested. Release: NTF activities were released from isolated bovine chromaffin cells upon cholinergic stimulation by a Ca^{++}-sensitive mechanism. Molecular characteristics: NTF activity residing in isolated bovine chromaffin granules was not blocked by antibodies to NGF and chromogranins A, B, C. After separation on HPLC molecular sieve columns an activity addressing CG8 neurons eluted at M_r = 22 Kd. Activities for CG8 and DRG8 neurons could also be enriched on DEAE sephacel ion exchange and heparin-sepharose affinity columns. Preliminary data from 'cell blots' (Carnow et al., J. Neurosci. 5, 1965, 1985) revealed CG8 neuron survival on 4 distinct bands with M_r = 24, 34, 55 and 95 Kd. 24 Kd is the molecular weight of CNTF isolated from rat sciatic nerve (Manthorpe et al., Brain Res. 367, 282, 1986). These results suggest (i) tissue and target neuron specificity with regard to NTFs residing in adrenal medulla and cortex, respectively, and their regulation during ontogeny, (ii) NTF release from chromaffin cells by a Ca^{2+}-dependent mechanism and, (iii) presence of a CNTF-like protein and another heparin-binding NTF, which, unlike CNTF, addresses DRG8 neurons. Pertinent to the latter may be our finding that chromaffin vesicle proteins, like several heparin-binding growth factors, trigger proliferation of cultured endothelial cells.

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444.4 **CILIARY NEURONOTROPHIC FACTOR IN EYE TISSUES OF AVIAN EMBRYOS TREATED WITH ALPHA-BUNGAROTOXIN** **H.J.L.Fryer*,S.D.Meriney, and G.Pilar. Dept Physiology and Neurobiology, U-42, Univ. Conn, Storrs, CT, 06268**

During development 50 % of the neurons in the ciliary ganglion die between embryonic day (E) 8 and 14. We have shown that all of the neurons survive this death period if alpha-bungarotoxin (BTX) is injected into developing chicken embryos. Since target derived survival factors are thought to be important in the regulation of neuronal death we investigated the effect of BTX administration on ciliary neuronotrophic factor (CNTF) activity in developing chicken eyes.

CNTF activity in eye extracts was quantified by a 24 hour culture assay as was previously described (Manthorpe,M.,*et al*, Brain Res.,230:295,1981). Confirming a previous report (Landa,K.,*et al*,Dev.Biol.,74:401,1980) we have found that CNTF activity in control eye extracts was initially low until E 11, after which it increased rapidly to peak at E 15. In BTX treated embryos CNTF activity increased early in development peaking at E11, which was then followed by a steady decline. At E11 the eye CNTF activity in BTX treated embryos was 2.5x greater than controls, and at E15 3x less than controls. To determine the tissues which were affected by BTX treatment, eyes from BTX-treated and control embryos were dissected into two fractions: the vascular choroid coat, and striated muscular ciliary body and iris. The early increase and later decrease seen in the eyes of BTX-treated embryos occurred in the choroid. No changes were detected in the ciliary body/iris tissues. BTX blocks neuromuscular transmission in the iris and ciliary body but does not affect transmission in the choroid. Thus there is no correlation between the the level neuromuscular transmission and concentration of trophic activities in this system.

Because a greater number of neurons survive and innervate their target tissues in BTX-treated embryos, the lower eye activity of E 15 embryos may be due an increase of retrograde transport of CNTF from the target tissues. To test this hypothesis the eyes of either contols or BTX-treated E 13 embryos were injected with colchicine, which blocks axonal transport. At E15 eye CNTF activity was assayed. CNTF activity in the eyes of both BTX-treated and control embryos was higher than uninjected eyes, which suggests that retrograde transport may account for the lower eye CNTF activity in E 15 BTX-treated embryos.

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444.5 A PUTATIVE TROPHIC FACTOR FOR CILIARY GANGLION NEURONS IN SKELETAL MUSCLE. N.I.Mundy* and A.W.Mudge* (SPON: J.G.R. Jefferys). MRC Developmental Neurobiology Prog., Dept. Zoology, University College London, London WC1E.

Previous work (A.W. Mudge and H.V. New [1986] Soc.Neurosci. Abstr. 300, 1) described a putative motoneuron trophic factor, intramuscular peptide (IMP), which is present in developing chick skeletal muscle. IMP is recognised by some antibodies directed to the amino terminal end of vasoactive intestinal peptide (VIP). IMP extracted from muscle elutes from a gel filtration column as two major peaks of molecular mass 20KD and 10KD. It is present in cultured chick myotubes and is secreted by them.

In the present study we have investigated the possibility that IMP might be a trophic factor for ciliary ganglion (CG) neurons. In the chick, the ciliary subpopulation of CG neurons are like motoneurons in that they are cholinergic and project to striated muscle. Firstly, immunohistochemistry for IMP was performed on the developing ciliary and iris muscle, the two targets for ciliary neurons. IMP was present in both of these sites during a restricted phase of development. In the ciliary muscle staining was first seen in myoblasts at E9; peak staining intensity was reached at E11 and E13 and staining was absent by E18; in the iris, staining was seen at E11 and E13. Neuronal death in the chick CG occurs between E8-E14 and so these findings are consistent with a potential role for IMP as a trophic factor during this period. Secondly, a 48 hour survival assay for E8-E9 chick CG neurons has been established in vitro. The dissociated cells are grown in a high potassium medium on a matrix produced by Schwann cells. We have confirmed that saline extracts of E8-E10 skeletal muscle support neuronal survival, whilst synthetic VIP does not. We are currently using affinity-purified antibodies to establish the role of IMP in supporting survival of these CG neurons.

444.6 DISTRIBUTION OF CALCITONIN GENE-RELATED PEPTIDE IN CHICK MOTONEURON POOLS. M.Tessier-Lavigne* and A.W. Mudge*(SPON: D.A. Brown). MRC Developmental Neurobiology Prog., Dept. Zoology, University College London, London WC1E.

Calcitonin gene-related peptide (CGRP)-like immunoreactivity has been observed in a subpopulation of motoneurons in chick spinal cord from embryonic day 6 (E6) onwards, and cultured chick muscle has been shown to express receptors for CGRP (New and Mudge, Nature 323:809-811, 1986; and C. Jennings and A. Mudge, unpublished observations). Further, some motoneurons stain intensely for CGRP, while others stain only weakly. In an attempt to obtain further clues regarding the function of this peptide, we have studied the identity of limb muscles innervated by $CGRP^+$ motoneurons in E10-E12 chicks.

This identification was performed in two ways. First, the location of $CGRP^+$ neurons, observed by immunohistochemistry in serial longitudinal or transverse cryostat sections of chick lumbo-sacral spinal cord (segments 23-30), was compared to the known location of motoneuron pools, as described by Landmesser (J.Physiol. 284:371-389, 1978) and Hollyday (J.Comp.Neurol. 194: 143-170, 1980). Second, motoneurons projecting to individual thigh muscles were retrogradely labelled in vitro (Landmesser, 1978) by injecting these muscles with tetramethyl rhodamine isothiocyanate (TRITC) conjugated to wheat germ agglutinin. $CGRP^+$ neurons within the $TRITC^+$ pools were identified by fluorescein immunofluorescence. This second procedure confirmed the identification of motor pools for thigh muscles obtained by the first method, and allowed us to quantify the number of $CGRP^+$ neurons in individual pools. Many $CGRP^+$ cells clustered to form longitudinally oriented columns surrounded by $CGRP^-$ cells. These columns were seen to correspond to motor pools, as follows. Virtually all cells in the sartorius motor pool and the motor pools innervating muscles of the dorsal shank showed intense staining, as did the majority of cells in the medial motor columns at the level of segment 23. A large number of motoneurons in a longitudinally-oriented column in the dorso-medial portion of the adductor pool also stained intensely. Less intense staining was observed in many of the cells innervating muscles of the ventral shank: as the motor pools for these muscles overlap we have not been able to determine whether the motor pools for any one of these muscles is completely $CGRP^-$. Few $CGRP^+$ cells were observed in the femorotibialis, iliofibularis, ischioflexorius and iliofibularis pools. To our knowledge, this is the first demonstration of biochemical differences between motoneurons projecting to different targets.

444.7 A SUBSTRATUM-BINDING FACTOR FROM MUSCLE THAT PROMOTES NEURITE OUTGROWTH FROM NEURONS OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEMS. T.H. Oh, G.J. Markelonis, T.L. Dion* and S.L. Hobbs*
Dept. Anatomy, Univ. Maryland Sch. Medicine, Baltimore, Maryland, 21201.

Spinal motor neurons depend upon muscle-derived trophic factors for their development and survival. Some circumstantial evidence suggested to us that the regulatory subunit of 3',5'-cyclic-adenosine monophosphate-dependent protein kinase (cAMP-dPK) type II might be involved in neuritic outgrowth from spinal neurons (Gurney, M.E., Nature, 307: 546, 1984; Held, I.R. et al., J. Neurosci., 3: 2054, 1983). In the present study, we tested a commercial preparation of cAMP-dPK for its neurite-promoting activity on chicken embyonic spinal neurons in culture. Commercial cAMP-dPK type II from skeletal and cardiac muscles elicited a significant neurite outgrowth from cultured neurons when the enzyme preparation was bound to polylysine-coated substrata; type I cAMP-dPK from skeletal muscle was ineffective. Neither cAMP-dPK type I nor type II had a significant effect on the survival of spinal neurons in culture. Type II cAMP-dPK also stimulated neurite outgrowth from chicken cerebral hemisphere neurons, dorsal root ganglionic neurons, ciliary ganglionic neurons and rat sympathetic ganglionic neurons in culture. However, as neither the purified regulatory nor catalytic subunits of cAMP-dPK type II had an effect on neurite outgrowth per se from cultured neurons, and as neurite-promoting activity was not correlated with ^{3}H-cAMP binding nor cAMP-dependent kinase activity, it seemed that neurite-promoting activity resided in a contaminant of the preparation. The neurite-promoting protein was partially purified from commercial cAMP-dPK type II by gel filtration on Sephadex G-200 followed by ion-exchange chromatography on DE-52 cellulose. SDS gel electrophoresis of the active protein fraction revealed a major protein band (MW, 50 kd) and several minor bands (e.g., MW 200 kd, 45 kd etc.). Western blot analysis and immunoprecipitation revealed that the neurite-promoting protein was distinct from laminin, nerve growth factor, neural cell adhesion molecule and fibronectin. Furthermore, this neurite-promoting protein was not retained on a heparin affinity column nor destroyed by heparinase. Our results demonstrate that a protein which co-purifies with the type II cAMP-dependent protein kinase of striated muscle stimulates neurite outgrowth from neurons of the central and peripheral nervous systems. Supported by the NIH (NS 15013-THO and NS 20490-GJM).

444.8 PURIFICATION OF A NEUROTROPHIC FACTOR FROM PERIPHERAL NERVES P.M. Richardson, M. Altares* and R.J. Riopelle. McGill University, Montreal and Queen's University, Kingston, Canada.

A purification scheme is reported for a molecule that supports chick ciliary neurons in tissue culture and is relatively concentrated in peripheral nervous tissue (Ebendal et al, Neurosci. Lett. 14, 91 (1979); Barbin et al, J. Neurochem. 43, 1468 (1984); Collins, Dev. Biol. 109, 255 (1985). Rabbit sciatic nerves are pulverized in a buffer with protease inhibitors and defatted with petroleum ether. The homogenate is then loaded on preparative and semi-preparative C18-silica columns, bioactivity eluting from the latter column with 55-60% acetonitrile in 0.1% trifluoroacetic acid. Despite 90-95% reduction in specific activity on simple exposure to acetonitrile, enough bioactivity remains after reverse-phase HPLC to monitor further purification. The next step in purification involves an ion-exchange column (Mono-Q) and elution with a gradient of ammonium acetate in water. Finally bioactive fractions are loaded on a C-4 silica column and eluted again with acetonitrile-trifluoroacetic acid. The yield from these procedures is in the order of 5 ng/gm of a product that is bioactive on chick ciliary neurons at concentrations below 1 ng/ml even after denaturation. The apparent molecular weight of the neurotrophic activity is approximately 15,000 on gel-exclusion HPLC. Because of the denaturation, the purification protocol is appropriate for biochemical rather than physiological studies.

444.9 ARE ALL NEUROTROPHIC FACTORS RETROGRADELY TRANSPORTED? R.A. Rush*, P. Smet* and I.K. Abrahamson* (SPON: C. Straznicky). Department of Physiology and Centre for Neuroscience, Flinders University of South Australia, 5042.

Neurotrophic factors are generally assumed to be soluble proteins taken up by nerve terminals and transported to the cell soma, where they act to allow neuronal survival. However it is not known if retrograde transport is an essential requirement for the action of all neurotrophic agents. Thus, ciliary neurotrophic factor covalently bound to nitrocellulose allows the survival and outgrowth of ciliary neurons (Carnow et al, J. Neurosci. 5:1965, 1985). Further indirect evidence to suggest that a factor for ciliary neurons is not transported is the demonstration that trophic activity does not accumulate in the iris after denervation (Ebendal et al, Neurosci. 16:181, 1985). In contrast, NGF which is transported, readily accumulates when retrograde removal is prevented by axotomy or colchicine (Korsching and Thoenen, J. Neurosci. 5:1058, 1985).

We have investigated whether a growth factor which supports sympathetic and ciliary neurons is transported in chick peripheral nerves. Expansor secundariorum and sciatic nerves were ligated for 24 hours and the nerve segments proximal and distal to the ligation were homogenised. Dose response curves of the homogenates were established using both sympathetic and ciliary neuronal bioassays. Distal segments contained more sympathetic neurotrophic activity than proximal segments, but this increase could be blocked by the addition of NGF antibodies to the bioassay. The remaining non-NGF activity, which supported both sympathetic and ciliary neurons, was distributed equally across the ligation. These findings demonstrate the presence of a growth factor distinct from NGF in peripheral nerve, which is apparently not transported. The mechanism by which this factor keeps neurons alive is currently being examined.

444.10 **CHARACTERIZATION OF INTERPHOTORECEPTOR MATRIX PREPARATIONS THAT PROMOTE PHOTORECEPTOR SURVIVAL IN RETINAL MONOLAYER CULTURES.** J. D. Lindsey, A. T. Hewitt and R. Adler, Wynn Center, Wilmer Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205

The survival of many peripheral neurons is specifically dependent on trophic factors present within the surrounding microenvironment (e.g. NGF and ciliary neuronotrophic factor). Although it is likely that many CNS neurons also require specific trophic factor support, their requirements have not been assessed because of the difficulty in recognizing specific types of CNS neurons in heterogeneous cultures. Recently developed cultures of chick embryo retinal neurons and photoreceptors have overcome this drawback. Cells in these cultures express many differentiated properties which allow for their unambiguous identification by morphological, immunocytochemical and autoradiographic techniques (rev. Adler, R. Prog. Ret. Res. 6:1, 1986). A possible source of putative trophic factors for photoreceptors is the interphotoreceptor matrix (IPM), a mixture of proteoglycans and glycoproteins localized between photoreceptors and the retinal pigmented epithelium. IPM preparations [isolated as described by A. Adler and K. Severin (Exp. Eye Res. 32:755, 1981)] were found to promote the survival of cultured photoreceptors by up to 400%, without affecting the survival of the multipolar neurons. This photoreceptor-specific response was not mimicked either by known growth factors, such as NGF or FGF (gifts of M. Manthorpe), or by Interphotoreceptor Retinoid-Binding Protein (gift of G. Chader), the best characterized IPM component. IPM preparations can exert their photoreceptor-survival promoting effect either when added to the medium, or when substratum-bound. The activity is nondialyzable, heat-labile and inactivated by pH $\leq$ 5.0 and $\geq$ 10.0. Activity can be recovered from Sephacryl S-200 gel filtration columns in two separate regions (K_{av}=0.12 and 0.41). Furthermore, it binds to DEAE-Sephacel from which it can be eluted with 0.25 M NaCl. This evidence indicates that an activity that specifically promotes photoreceptor survival in vitro is associated with macromolecular components of the IPM. Further purification of the activity and generation of antibodies will be needed in order to establish whether this activity regulates photoreceptor survival in vivo.

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444.11 PARTIAL PURIFICATION AND CHARACTERIZATION OF A NEURITE-PROMOTING FACTOR FROM THE REGENERATING GOLDFISH OPTIC NERVE. C.G. Caday*, P.J. Apostolides*, L.I. Benowitz, N.I. Perrone-Bizzozero, and S.P. Finklestein. Mailman Research Center, McLean Hospital, Belmont, MA; and Massachusetts General Hospital, Boston, MA.

Unlike most pathways of the mammalian central nervous system (CNS), many CNS pathways of lower vertebrates can regenerate throughout life, a capacity that may be due to the secretion of neurite-promoting molecules from the injured CNS. In previous studies (Finklestein, S.P., et al., Brain Res., in press) we found that conditioned media (CM) from the regenerating goldfish optic nerve (ON) (but not from the intact ON, or intact or injured ON of mature rats) promoted marked neurite-outgrowth from dissociated mammalian (rat) embryonic cerebrocortical and hindbrain neurons in serum-free, neuron-enriched culture. Neurite-promoting activity was substrate bound, and the active factor(s) appeared to be one or more protein species, due to properties of non-dialyzability, and heat and trypsin sensitivity.

In the current study, we have partially purified and characterized a neurite-promoting factor from the regenerating goldfish ON using membrane filtration, ion-exchange, and gel-filtration chromatography. Neurite-promoting activity was retained by an ultrafiltration membrane of pore size 10,000 (Centricon-10), bound strongly to an anion exchange column (DEAE-cellulose) at physiological salt concentrations, and was eluted from this column at 0.3 - 0.4 M NaCl. Active fractions were then applied to a gel-filtration column (Biogel P-100), and the major peak of neurite-promoting activity was eluted at an apparent molecular weight of 25 ± 5 kD. This acidic 25 kD protein from the regenerating goldfish optic nerve appears to be distinct from other characterized molecules with known neurite-promoting effects on mammalian CNS neurons.

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444.12 SOLUBLE PROTEINS FROM RAT OLFACTORY BULB SUPPORT THE SURVIVAL AND DIFFERENTIATION OF CULTURED BASAL FOREBRAIN NEURONS. M.P. Lambert*, T. Megerian*, G. Garden*, and W.L. Klein. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

Basal forebrain cells from E15-16 rat cultured in defined medium die within several days of plating. Addition of olfactory bulb (OLB) extract to defined medium causes enhanced survival and cellular differentiation. Growth in the presence of extract causes increased neurite production, aggregation of the cells, and formation of thick, axon-like neurites between the aggregates. The presence of both cholinergic neurons and glial cells in the culture is shown by acetylcholinesterase and GFAP-positive staining, respectively.

Response of the culture to OLB extract depends on plating density and time of exposure. Cells plated at a density of less than 4,000 cells/square cm show no response to extract at 0.1 mg/ml, whereas cells plated at 10 times that amount respond to extract as described. Constant exposure to extract appears to be necessary for maximum survival times, since cells with 1-5 days exposure to extract survive only 1-2 days after extract removal.

Concentrations of extract between 0.002 and 0.1 mg protein/ml increase the percentage of cell survival and neurite production, reaching a plateau at higher doses and having no effect at lower doses. The active factor(s) in OLB extract is sensitive to both heat and trypsin treatment. Neither dialysis or repeated freezing and thawing of the extract affects its ability to cause a response. Pre-treatment of the substrate with the extract does not elicit any increase in cell survival or neurite extension. Neither NGF, laminin, or other additions to the culture, such as BSA, pyruvate, or ascorbate, cause responses similar to OLB extract. Initial fractionation of the extract utilizing a Sephadex G-75 column indicates that one active factor is a molecule with molecular weight greater than 66 kD. Medium conditioned by slices of adult OLB also increases survival and neurite production of basal forebrain cells, supporting the hypothesis that the trophic activity is secreted by CNS tissue. (Supported by NIH grant NS23348 to WLK.)

444.13 CHEMICAL AND BIOLOGICAL CHARACTERIZATION OF A STRIATAL-DERIVED NEURONOTROPHIC FACTOR (SDNF): COMPARISON WITH OTHER NEURONOTROPHIC FACTORS. G. Ferrari*, C. Soranzo*, S.D. Skaper, D. Benvegnù*, G. Toffano, A. Leon and L. Callegaro*. (SPON: M. Vitadello). Fidia Research Laboratories, 35031 Abano Terme (PD), Italy.

We have previously reported that bovine striatal extracts are capable of increasing the survival and maturation of fetal mouse mesencephalic neurons maintained in serum-free culture conditions, (Dal Toso et al., Soc. Neurosci. Abstr., Vol.12, Part 2, p.1100, 1986). This biological activity is associated with a fraction whose main component is a basic protein of approximately 14 Kd. Since several defined growth factors have been reported to possess similar effects on cultured CNS neurons, we evaluated the chemical characteristics, as well as the biological activity of SDNF in comparison to Nerve Growth Factor (NGF) and basic Fibroblast Growth Factor (bFGF). SDNF, like NGF, is able to promote neurite regeneration in NGF-primed PC12 cells; however, unlike SDNF, NGF is totally without effect on the mesencephalic neurons. In contrast, both SDNF and bFGF display similar activities toward the mesencephalic neurons. When applied to a heparin column, the 14 Kd protein of the most pure SDNF fraction shows a different elution profile with respect to bFGF. Experiments are currently in progress to further compare SDNF to bFGF, utilizing chemical and immunological techniques, as well as the biological activities addressing a variety of neuronal and non-neuronal cell types.

444.14 AFFINITY CHROMATOGRAPHY OF NEUROTROPHIC AND NEURITE-PROMOTING FACTORS FROM INJURED BRAIN. D.L. Needels, M. Nieto-Sampedro, and C.W. Cotman. Department of Psychobiology, University of California, Irvine, CA 92717.

The tissue surrounding the site of a brain lesion contains increased levels of trophic factors for a variety of neurons. In this study, two group-specific affinity columns were tested for their utility in the purification of trophic factors from injured brain. Heparin-Sepharose has proven useful in the purification of a variety of growth factors (and other proteins) containing heparin binding sites. Blue-Sepharose has been used extensively in the purification of nucleotide-requiring enzymes, as well as other proteins containing large hydrophobic pockets (including several trophic factors).

For each column, ~10 rats underwent bilateral suction ablation of entorhinal/occipital cortex. The animals were sacrificed 12 days later, the tissue surrounding the wound cavity was dissected out, and extracts were prepared by homogenization in 4 volumes of ice-cold 20 mM HEPES buffer, pH 7.2. After centrifugation (70,000 x g x 30 min), the soluble fraction (~18 ml) was applied to either a 1.5 x 2.5 cm Heparin-Sepharose column or a 2.5 x 35 cm Blue-Sepharose column equilibrated with 20 mM HEPES, pH 7.2. Retained proteins were eluted with a linear NaCl gradient. Fractions were collected and assayed for both survival-promoting (neurotrophic) and neurite-promoting (sprouting) activities using cultures of chick ciliary ganglion neurons.

A portion of both types of bioactivity was retained by the two affinity columns. By adjusting the NaCl concentration during the wash and gradient, the retained fractions could be divided into 3 general classes corresponding to weak, strong, and very strong interactions with the column matrices. Trophic activity was found in all 3 classes eluted from Heparin-Sepharose. Some of the protein retained at low ionic strength binds through non-specific ionic interactions with the negatively-charged heparin. This is probably not the case with the trophic factors described here since all of the bioactivity binds to positively-charged DEAE-cellulose columns. The most tightly bound activity required over 1M NaCl for elution, comparable to the conditions used to elute growth factors (such as fibroblast growth factor) that contain genuine heparin binding sites. On the Blue-Sepharose columns, much of the activity was also found in the most tightly bound fractions. Only a small proportion of the total protein was found in this fraction, resulting in a very good purification of bioactivity.

A series of step elutions was designed for each type of column so that the classes of bioactivity could be separated without requiring a gradient or concurrent assay of individual fractions. These protocols can be used to separate and purify neurotrophic and neurite-promoting factors from injured brain, and to identify those factors which increase following injury or denervation.

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444.15 GROWTH OF NEURONAL CELLS FROM HUMAN FETAL BRAIN IN A CHEMICALLY DEFINED MEDIUM. G. Haselbacher*+, P. Groscurth*++, U. Otten+++, H. Vedder*+++, U. Lutz*+, P. Sonderegger+, A. Bulatko*§, N. Greeff*§, and R. Humbel*+ (SPON: E. Sommer). + Dept. of Biochemistry, ++ Dept. of Anatomy, § Dept. of Physiology University of Zürich, +++ Dept. of Pharmacology, Biocenter, University of Basel (Switzerland).

Brain fragments from human fetuses (legal abortions, 12th - 16th week of gestation) were kept frozen in liquid nitrogen. Dissociated cultures were established in a chemically defined medium from which insulin had been omitted. Instead, the medium was supplemented with either Insulin-like growth factor (IGF) I, II, NGF or NGF and IGF II combined. The cultures were kept for 4 weeks during which time they were characterized by light microscopy, transmission and electron microscopy as well as by immunocytochemical methods. The proportion of glial acidic protein (GFAP) positive cells gradually decreased from an initial 80% to about 10 - 20%, while the neuronal cells increased accordingly. Thus 3 week old cultures contained essentially only neurons. Autoradiography with ^{3}H-thymidine also showed neuronal cell division.

Using the patch clamp technique, voltage dependent Na^{+} channels were recorded. Furthermore these cultures produced neuropeptides such as substance P and substance K, somatostatin and express cholinergic properties as indicated by choline acetyltransferase activity.

In conclusion, we have established the conditions to grow neuronal cells from human fetal brain in a chemically defined medium. The use of frozen material allowed pooling of tissue of comparable gestational age and to carry out relatively large scale experiments. Such cultures may serve as a useful tool to address a variety of questions on human brain differentiation including neuronal development, the actions of neurotrophic factors as well as neurotoxicity screening.

TROPHIC INTERACTIONS II

445.1 THE LAMININ BINDING PROPERTIES OF RAT PC12 CELLS. V. Nurcombe*, D. Edgar*, R. Timpl*, and H. Thoenen. MPI für Psychiatry, D-8033 Martinsried, FRG (SPON: H. Holländer).

The basement membrane protein laminin has been shown to potentiate the survival and neurite outgrowth of target neurons of both NGF and brain-derived neurotrophic factors (D. Edgar et al., EMBO J., 3:1463, 1984; R.M. Lindsay et al., Dev. Biol., 112:319, 1985). It has been further shown that a specific domain of the laminin molecule including the end of the long arm and the heparin-binding globule is associated with its effects on chick sympathetic neurons (D. Edgar et al., EMBO J., 3:1463, 1984). This laminin fragment has also been shown to selectively increase the levels of those enzymes specifically involved in the catecholamine biosynthetic pathway in calf adrenal chromaffin cells (A. Acheson et al., J. Cell Biol., 102:151, 1986). The aim of the present study was to document the effects of laminin fragments on PC12 cells, prior to the characterisation of laminin-binding proteins from cell membrane preparations.

Laminin and laminin fragments prepared from EHS sarcoma (R. Timpl et al., J. Biol. Chem., 254:9933, 1979) were radioiodinated, and ligand-binding studies to intact, suspended cells at 0°C performed. Subsequent Scatchard analysis revealed a single glass of high affinity receptors with a K_D of $3x10^{-9}$M. Of the 6000 binding sites per cell, almost all bound the end of the long arm. Whole laminin binding could be blocked with fragments corresponding to the long arm of the molecule including the heparin-binding domain, but not by other fragments including those derived from the short arms. The active fragment was also able to reproduce the potentiating effects of whole laminin on PC12 cell neurite outgrowth, enzyme induction, and response to NGF. It is concluded that, as is the case for neurons, the end of the long arm mediates the binding of laminin to PC12 membranes.

445.2 PURIFICATION AND CHARACTERIZATION OF NEURONAL PRECURSOR CELLS FROM NEONATAL RAT ADRENAL GLANDS. D. Roufa, L. Kahn*, S. Rapp*, W. Engleman*, and K. O' Malley[1]. *Searle Research & Development, Chesterfield, MO. 63198, [1]Washington Univ. Sch. of Med. , St. Louis, MO 63105.

Adrenal medullary cells and sympathetic neurons are neural crest derivatives that become committed to express endocrine-like (chromaffin cells) or neuronal-like (sympathetic neurons) phenotype depending upon the environment in which they reside and the repertoire of trophic molecules to which they are exposed. A subpopulation of chromaffin cells retain the ability to express neuronal phenotype in response to Nerve Growth Factor (NGF). Since neuronal cells are post-mitotic, the availability of neuronal precursor cells and the understanding of the environmental factors that control expression of neuronal phenotype has important implication for successful neuronal regeneration and functional recovery in the adult central and peripheral nervous system. Adrenal medullary cells obtained from embryonic, neonatal or adult animals and grown in culture have been shown to express neuronal-like morphology when grown in the presence of NGF. A monoclonal antibody that recognizes NGF receptors (NGFR) (IgG-192, gift from Dr. Eugene Johnson) was used to label the potential NGF responsive cells. A subpopulation of NGFR positive cells was identified. Both the number of stained cells and the intensity of staining increased following exposure to NGF. In order to purify the subpopulation of NGFR bearing cells, adrenal glands were removed from postnatal day 7 rats, the adrenal medulla was separated from the adrenal cortex under a dissecting microscope, enzymatically dissociated and pre-incubated with NGF (100 ng/ml) for 48 hours at 36°C in humdified chamber with 5% CO_2 in air. Following preincubation, the cells were labeled with the anti-NGFR monoclonal antibody and visualized by a fluorescence detection system consisting of biotinylated horse anti-mouse IgG and avidin conjugated fluorescein. Cells were sorted based on fluorescence intensity and forward light scatter. Positively fluorescent cells as well as non-fluorescent cells were collected separately under viable and aseptic conditions and plated on collagen coated microtiter plates, or frozen in liquid N_2 for further studies. Approximately 3-10% of the sorted cells were NGFR positive and better than 90% of the sorted NGFR positive cells expressed neuronal morphology when grown in the presence of NGF. Further characterization indicates that these cells contain tyrosine hydroxylase (TH) as determined by immunocytochemistry using anti-TH antibodies and by hybridization studies using TH mRNA as the probe. In summary, we have purified a subpopulation of neuronal precursor cells from adrenal medulla that are NGF responsive and resemble sympathetic neurons in their neurotransmitter choice.

445.3 IN VITRO STUDIES ON THE ROLE OF ACTIVITY IN THE EXPRESSION OF A SYNAPTIC VESICLE PROTEIN IN RAT SUPERIOR CERVICAL GANGLION. K. M. Linderman* and K. F. Greif. Bryn Mawr College, Bryn Mawr, PA 19010.

In vivo studies have shown transsynaptic factors are involved in the regulation of expression of a 65 kilodalton integral membrane protein (SV) of synaptic vesicles. Neonatal deafferentation of the superior cervical ganglion (SCG) produces a reduction in SV levels, a result which seems to be supported by pharmacological studies. The ganglionic blocking agent, chlorisondamine, administered to neonates twice daily for two weeks reduces SV levels by approximately 30% (Greif and Linderman, Soc. Neurosci. abstr. 12: 1109, 1986). However, weight reductions and other possible toxic side effects limit the usefulness and clarity of these experiments. Therefore, an in vitro SCG culture system was adopted to investigate the role of activity in the regulation of SV expression. SV levels were quantified by radioimmunoassay.

In SCG explants, cultured in defined medium (Kessler et al, Neurosci. 9: 309, 1983) SV levels doubled in the first two days and gradually declined over two weeks, but never fell below levels expressed in two day old SCG. However, total protein content of the ganglia also declined rapidly in the first two days in culture, so that SV levels standardized for ganglion size rose approximately 60% and were equal to initial levels after 14 days in culture. These results may reflect non-neuronal cell death since the medium used does not support survival of these cells. The results thus suggest that removal to culture prevents normal postnatal increases in SV in the SCG.

Activity of cultured SCGs was modified by the addition of the depolarizing agent, veratridine (VER, 10^{-5} M), potassium (30mM), and/or tetrodotoxin (TTX, 10^{-7} M) to the medium. Highly active cultures (+VER) showed significant increases (>150%) after 24 hours of treatment and levels remained high with sustained treatment as long as 14 days. This induction of SV was reversed by concurrent administration of TTX with VER. High K+ medium also caused smaller increases in SV levels; increases were not completely reversed by TTX.

These in vitro studies support the in vivo findings that activity plays a role in the regulation of expression of SV in the SCG during postnatal development. Other transsynaptic factors involving target interactions or trophic factors may also be important.

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445.4 NORADRENERGIC CHARACTER OF CHICK SYMPATHETIC NEURONS IS ENHANCED BY NON-NEURONAL CELLS. A.R. Wakade and S.V. Bhave.* Dept. of Pharmacology, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203.

Noradrenergic character of sympathetic neurons (SN) derived from peripheral sympathetic ganglia of 10-12-day-old chick embryo and maintained in vitro under various culture conditions was examined by measuring norepinephrine (NE) content and ^{3}H-NE uptake. SN were cultured in a serum-containing or serum-free medium as described before (Wakade et al., Exptl. Cell Res. 140, 71, 1982). SN supported in culture in a serum-free or serum-containing medium showed almost equivalent uptake of ^{3}H-NE (141 x 10^{-3} CPM/dish). A significant uptake (181 x 10^{-3} CPM/dish) was observed when SN were co-cultured with chick heart or liver cells in the absence of NGF for 3 days. Addition of NGF to these co-cultures caused a marked facilitation of ^{3}H-NE uptake (373 x 10^{-3} CPM/dish). Uptake of ^{3}H-NE was facilitated when SN were co-cultured with chick skeletal muscle cells; however, the facilitation was evident only in the presence of NGF. When the SN were co-cultured with sensory neurons there was again a marked facilitation of uptake in the absence as well as the presence of NGF. The facilitatory effects of heart or liver cells on the uptake of ^{3}H-NE by SN was persistent for 6 days only in the presence of NGF. Culturing of SN in a medium conditioned by either of these target cells also resulted in a facilitation of the uptake. SN initially selected in culture by NGF in regular medium and then exposed to heart-conditioned medium for 3 days exhibited an increase in ^{3}H-NE uptake. There was approximately 5- to 6-fold increase in NE contents of SN co-cultured with non-neuronal cells, and about 2-fold increase when SN were grown in a medium conditioned by the non-neuronal cells. The NGF-deprived neurons had very low activity of choline acetyltransferase (ChAT); addition of serum along with NGF resulted in a marked increase in ChAT. A significant increase in ChAT was observed when SN were co-cultured with heart or liver cells, but not skeletal muscle cells. ChAT activity was not elevated when SN were grown in a medium conditioned by the heart cells. These results suggest that various types of cells secrete trophic factor(s) that specifically enhance the noradrenergic character of the NGF-dependent SN of the chick embryo.

445.5 SYNERGISTIC EFFECTS OF MUSCLE AND CENTRAL GLIAL DERIVED TROPHIC FACTORS ON THE SURVIVAL OF EMBRYONIC CHICK MOTOR NEURONS IN CULTURE. U. Dohrmann* and F.S. Walsh (Spon: J. Brockes). Institute of Neurology, Queen Sqare, London WC1, U.K.

Identified and isolated spinal motor neurons from 6-day-old chick embryos are unable to survive in culture unless provided with a soluble trophic factor from embryonic muscle (A.L. Calof and L.F. Reichardt, Dev. Biol. 106:194-210; U. Dohrmann et al, Dev. Biol. 118: 209-221). However, some motor neurons, identified by retrograde labeling with rhodamine, will survive in mixed spinal cell cultures in the absence of muscle extract. This survival promoting activity is a protein(s) secreted by glial cells from the central nervous system (CNS), and its production is enhanced by factors known to stimulate the proliferation of glial cells such as the glial growth factor GGF (G.E. Lemke and J.P. Brockes, J. Neurosci. 4:75-83).

At optimal concentrations, the CNS derived trophic activity can support the same number of cultured motor neurons as muscle extract. However, the two trophic activities interact in a synergistic manner: A) The CNS glial derived activity potentiates that of muscle to decrease its ED_{50} by an order of magnitude. B) Motor neuron survival seen in the presence of both factors is more than the sum of their individual activities.

A survival promoting activity similar to that from CNS glial cell conditioned medium is also detected in extracts from both embryonic and adult spinal cord and brain which suggests that the CNS derived trophic activity might play a role in the maintenance of adult motor neurons.

As a series of defined growth and survival factors present in the CNS (nerve growth factor, brain derived growth factor, acidic and basic fibroblast growth factor) have no effect on motor neuron survival, then we conclude that the molecule responsible for the motor neuron survival promoting activity of the CNS is probably a previously undefined factor.

Supported by the International Spinal Research Trust, London.

445.6 ANALYSIS OF A NEURITE OUTGROWTH FACTOR FROM DENERVATED MUSCLE EXTRACT WHICH EXHIBITS AN AGE-RELATED INCREASE IN POTENCY IN VITRO. J.L. Rosenheimer and D.O. Smith. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

Age-related changes at the neuromuscular junction are manifest as a decline in the number of nerve terminals and sprouts per end plate. Following denervation, nerve terminal sprouting within muscles from aged rats is virtually non-existent. These phenomena suggest that there may be an age-associated change in the normal trophic interactions between muscle and nerve.

To test whether these observations are due to an age-related change in some muscle-derived nerve sprouting factor, muscle extract from both limbs of 10- and 25-mos unilaterally denervated (denervated and contralateral) and unoperated (control) rats, was prepared and assayed in vitro for its effect on neurite outgrowth from a population of enriched motoneurons. Cells were plated on collagen gel-coated 16-mm wells containing Dulbecco's modified medium, defined supplements, and muscle extract ranging from 0 to 1 mg protein per ml medium. After 72 hr in culture the cells were fixed and photographically examined for neurite outgrowth.

A significantly greater percent of cultured neurons exhibited neurite outgrowth when coincubated with muscle extract from either limb of the 25-mos denervated rats compared to cells coincubated with 25-mos control muscle extract or extract from 10-mos denervated, contralateral, or control muscles (15.0%±0.2% vs. 9.6%±0.4%, respectively). In addition, extract from muscles of either limb of the 25-mos denervated rats was similar in potency to that from 10-mos denervated muscle only, all of which were 1.9±0.1x as potent as extract derived from 25-mos control or 10-mos contralateral or control muscles. Therefore, unlike previous results obtained from culture systems supplemented with serum, it appears that muscles from aged rats exhibit a more profound response to a challenge such as denervation compared to those from younger rats.

Preliminary attempts have been made to characterize the factor. Although SDS-PAGE reveals that soluble laminin is present in the extract, the activity is not immunoprecipitated with antisera to laminin, nor will coincubation with purified laminin produce neurite outgrowth. Furthermore, extract must be coincubated with the neurons to produce neurite outgrowth, indicating that the factor is soluble. Finally, ammonium sulfate precipitation separates the effect of the extract into neurite outgrowth activity (25-50% ammonium sulfate fraction) and survival activity (50-75% fraction). Supported by NIH grants AG05340 (JLR) and AG01572 (DOS).

445.7 ORGANOTYPIC ROLLER TUBE CULTURES OF RAT SKELETAL MUSCLE AND SKELETAL MUSCLE COCULTURED WITH SPINAL CORD. S. Ishimoto* and J.R. Delfs, William P. Arnold Pain Treatment and Research Center, New England Deaconess Hospital, and the Charles A. Dana Research Laboratories, Departments of Neurology, Beth Israel Hospital and Harvard Medical School, Boston, MA 02215.

We have previously reported (Delfs and Saroff, Soc.Neurosci.Abstr. 36:145, 1986) the culturing of rat spinal cord by the roller tube technique of Gahwiler (Neuroscience 11: 751, 1984). Using this technique, we have established cultures of neonatal rat skeletal muscle fibers alone and cocultures of rat skeletal muscle fibers with transverse slices of neonatal rat spinal cord.

When teased skeletal muscle fibers were cultured alone, many of the explanted muscle fibers remained histologically unchanged, with myoblast outgrowth or myotube formation being seen only rarely (<10%). In those rare cultures of muscle alone in which some regeneration was observed, development progressed only to the myotube stage, and in no case was development into mature muscle fibers observed.

On the other hand, when explanted muscle fibers were cocultured with transverse slices of spinal cord, regeneration of explanted skeletal muscle fibers occurred much more often (>40 %). In many of the cocultures, but in none of the cultures of muscle alone, the formation of nearly mature muscle fibers with sponataneous contractions was observed within 1-3 weeks.

Thus, we have demonstrated (1) that skeletal muscle can be grown using the roller tube technique of Gahwiler, (2) that cocultures of skeletal muscle with spinal cord can be established and maintained, and (3) that in vitro skeletal muscle regeneration is enhanced by the presence of cocultured spinal cord in these organotypic cultures.

(Supported in part by a grant from the Muscular Dystrophy Association.)

445.8 DOES TESTOSTERONE PREVENT AXOTOMY-INDUCED NEURONAL DEATH IN PREPUBERTAL RATS? Wan-hua Amy Yu. Department of Anatomical Sciences, City University of New York Medical School, City College, New York, New York 10031.

Following axotomy, a few cranial nerve motoneurons degenerate and die. We have shown previously, in the hypoglossal and facial motor nuclei of the adult rat, that a 25% (in females) and 15% (in males) reduction in the neuronal pool occurs after nerve transection; and that administration of testosterone to females attenuates such cell loss (Yu, W.H.A., Anat. Rec., 218:152A, 1987). Testosterone may act directly on neurons themselves or indirectly via other factors such as trophic substances derived from post-synaptic targets that alter neuronal metabolism. Since in adult rats motoneurons and their target muscle contain androgen receptors (Yu, W.H.A. and McGinnis, M.M., J. Neurosci., 6:1302, 1986), it is difficult to pinpoint the primary site of androgenic stimulation. In contrast, testosterone treatment of 2- and 3-week-old rats fails to accelerate regeneration of the hypoglossal nerve (Yu, W.H.A., Brain Res. Bull., 13:667, 1984). Motoneurons in neonates do not accumulate androgen in their cell nuclei (Breedlove, S.M., Soc. Neurosci. Abstr., 12:1220, 1986). These data imply the absence of androgen receptors in early postnatal rats. In contrast, skeletal muscle is endowed with steroid receptors at 3 days postnatal age (Tremblay, R.R. et al., Steroids, 29:185, 1977). The presence of androgen receptors in muscle but not in motoneurons of young rats provides a model to ask whether testosterone acts on neurons or muscle to promote neuronal survival. We report that testosterone prevents death of axotomized neurons in young rats as in adults.

Twenty-day-old female rats were anesthetized, the right hypoglossal nerve transected proximal to its bifurcation, the left facial distal to its exit from the stylomastoid foramen. After surgery, rats were divided into experimental (EXP) and control groups. The EXP group was injected intramuscularly with 0.5 mg testosterone propionate (TP) in oil twice weekly for 4 post-operative weeks and once a week thereafter. The control group received the oil vehicle. Five weeks after nerve section rats were perfused intracardially with 1% paraformaldehyde - 1.25% glutaraldehyde in phosphate buffer. Cell counts were made on serial paraffin sections stained with cresyl violet. Neuronal number in the nucleus of the lesion side was expressed as the percentage of the intact side.

Results indicate that TP treatment increases neuronal survival to 95% as compared to 80% in the control group. Thus, survival of axotomized neurons is probably enhanced as a result of interaction of the hormone with muscle. However, the possibility of hormonal action directly on neurons cannot be ruled out until the ontogeny of androgen receptors in motoneurons is known.

Supported by PSC-CUNY Research Award.

445.9 PERIPHERAL NERVE IMPLANT EFFECTS ON SURVIVAL OF RETINAL GANGLION LAYER CELLS AFTER AXOTOMY INITIATED BY A PENETRATING LESION. J.E.Turner, J.R.Blair and E.T.Chappell. Dept. of Anatomy, Bowman Gray Sch. of Med., Wake Forst Univ., Winston-Salem, NC 27103

Recent studies have demonstrated that adult rat retinal ganglion cells (RGC) are also included among the list of successful populations of CNS neurons able to regenerate long distances through the PNS bridge and even into a target tissue, the superior colliculus. In addition, we have recently reported the development of a model that will help provide additional information concerning possible effects of the peripheral nerve on the lesioned retina. (Turner, J.E. and J.R.Blair, Devel. Brain. Res, 26: 91-104, 1986). A lesion paradigm involving a small penetrating incision made through the sclera, choroid and retina of the adult rat eye creates a unique environment for the study of the damaged retina. More specifically, the dependable formation of a small focal retinal lesion makes this an ideal model for the determination of conditions that may stimulate retinal regeneration, wound repair and/or cell survival. Our previous studies have indicated that the placement of a desheathed peripheral nerve implant (PNI) into the lesioned adult rat eye: (1) initiated the rescue of retinal tissue that would normally die due to trauma; and (2) maintained the thickness and organization of most retinal layers adjacent to the lesion site, particularly the retinal ganglion cell layer (RGL). We report in the present work that a more detailed analysis of the RGL revealed a significant increase in the survival of axotomized neurons within this layer in the presence of a PNI over an 8 week post implantation period. More specifically, there was a 60% loss of control RGL cells axotomized by the lesion 3 weeks previously in contrast to only a 30% loss in those tissues exposed to a PNI. Additional RGL cell size distribution studies point to the fact that the PNI appeared to affect the increased survival of cells $\geq$ 70 μm^2. Also the PNI effect demonstrated a specificity of response when tested against the influence of a tendon or suture implant. Most interestingly, through the use of a double lesion paradigm, it was determined that the "rescue" effect in the RGL could be influenced over a distance possibly by a putative diffusable factor(s) elaborated or initiated by the PNI.

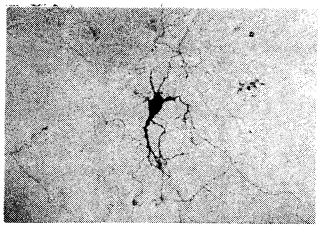
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445.10 Culturing Dissociated Adult Serotonergic Neurons on Fetal Hippocampal Neurons. E.C. Azmitia, P.M. Whitaker-Azmitia & M. Segal; Dept. Biology, WSCNS, New York University, NY NY 10003; Dept. Psychiatry, SUNY, Stony Brook NY; and Isotope Dept., CNS, Weizmann Inst., Rehovet, Israel

Co-culturing dissociated mesenephalic (MES) cells with hippocampal (HIP) neurons results in a marked stimulation of the survival, the high-affinity uptake of ^{3}H-5-HT and neurite outgrowth of 5-HT neurons (Azmitia & Whitaker-Azmitia, Neuroscience 20,47 1987). The trophic effect exerted by the target cells on serotonergic neurons has been demonstrated by transplanting fetal MES cells into the adult HIP. Adult MES transplanted into adult HIP did not survive. However, co-transplantation of adult MES and fetal HIP into the adult brain did result in the survival and maturation of transplanted adult 5-HT neurons (Azmitia in "Cell and Tissue Transplantation into The Adult Brain" NYAS, 1987). We now report that dissociated adult serotonergic neurons can be cultured when plated on cultured fetal hip neurons.

Fetal HIP neurons were dissected from a 18-D rat fetus and plated on poly-lysine coated plates as previously described (Segal, J. Neurophysio. 50, 1249 1983). At the first media change (3DIC), FUDR was added to suppress glia growth. HIP cultures of 3 day, 2 week or 1 month were used. Mes cells from fetal (D-13), neonate (PN-7) and adult (2 months) were dissociated in ice-cold D-1 solution by gentle re-pipetting. Complete neuronal media with 10% FCS and 10% HS was added to the dissociated MES cells and then plated on the HIP cultures, or on poly-lysine plates alone or as a co-culture with Hip cells (D-18). The media was changed to an FUDR containing CNM at 3DIC and 2 days later to CNM with 10% HS. At the end of 8DIC, pargyline (10^{-5}M) and L-tryptophan (10^{-5}M) were added to the cultures for one hour, the cultures fixed in ice-cold 4% paraformaldehyde in 0.1M phosphate buffer (PH 7.2) containing 5% sucrose. The cells were immunocytochemically stained for 5-HT using the ABC Kit with DAB as substrate. Fetal serotonergic neurons grew well in all conditions. Neonatal cells grew best on 2 week and 1 month HIP cultures. Adult serotonergic neurons were only observed on the 1 month HIP culture (see Figure).

CULTURED ADULT 5-HT NEURON



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445.11 NGF AND T_3 INCREASE ACTIVITY OF CHOLINERGIC ENZYMES IN CULTURED SEPTAL SLICES B.H. Gähwiler, A. Enz* and F. Hefti, Brain Research Institute, University of Zürich, Zürich; Preclinical Research, Sandoz Ltd., Basel, Switzerland and Department of Neurology, University of Miami, FL33101

Septal slices derived from 6-day-old rats were cultured either alone or together with hippocampal slices. Choline acetyltransferase (ChAT) determined after 3-4 weeks in vitro averaged 6.1 in septal monocultures and 10.8 pmol/min.µg protein in co-cultures. Exposure to nerve growth factor (NGF) and triiodothyronine (T_3) resulted in concentration-dependent increases in the activity of ChAT. NGF consistently enhanced ChAT activity up to 20-fold, whereas T_3-induced effects reached a maximum (5-fold increase) at a concentration of 50 ng/ml and declined with higher concentrations. Equivalent effects were observed in both in vitro preparations. Thyrotropin releasing hormone (up to 1 µg/ml) was without any effect on the activity of ChAT. The actions of NGF and T_3 were additive. In concentrations which had no influence on cholinergic enzymes per se, T_3 still enhanced the stimulatory effect of NGF. All actions on ChAT-activity were paralleled by similar increases in acetylcholinesterase activity. Antibodies to NGF failed to alter basic enzyme activity and T_3-induced effects, but completely abolished the action of NGF in mono- as well as co-cultures.

The biochemical observations were consistent with histochemical findings. In septal cultures, application of NGF and T_3 strongly enhanced the intensity of staining for AChE and ChAT without inducing radial outgrowth of fibers from the slices. In co-cultures, both substances increased the number of cholinergic fibers that invaded the hippocampal tissue.

445.12 MORPHOLOGICAL AND BIOCHEMICAL EFFECTS OF NERVE GROWTH FACTOR AND MONOSIALOGANGLIOSIDE GM1 ON SEPTAL CELLS IN CULTURE. R.L. Kenigsberg, D. Maysinger and A.C. Cuello. Department of Pharmacology and Therapeutics, McGill University, 3655 Drummond St. Montreal H3G 1Y6 Quebec, Canada.

The effects of nerve growth factor (NGF) and monosialoganglioside GM1 alone and in combination were examined in primary dissociated septal cells in culture. Morphological and biochemical parameters were monitored after various time periods of exposure to these agents.

Primary dissociated septal cells were prepared from rat fetal (E 17) septum. The cells were seeded at low (1.5×10^5 cells/sqcm) and high (3×10^5 cells/sqcm) densities on poly-l-lysine coated culture dishes. The culture media did not contain any metabolic inhibitors therefore providing us with a mixed neuronal-glial cell population.

The percentage of surviving neurons was determined immunohistochemically with the use of a monoclonal antibody raised against neurofilaments (Lawson, S.N. (et al.,) J.Comp. Neurol.228,263,1984). In addition, the cholinergic neurons were identified with the use of monoclonal antibodies raised against choline acetyltransferase (ChAT) (Eckenstein, F. and Thoenen, H., EMBO J,1:363,1982). The effects of NGF and GM1 on fiber outgrowth, neuronal survival and nerve growth factor receptor distribution was investigated immunohistochemically (Chandler, C.E. et al. J.Biol.Chem.259:6882,1984) both at the light and electron microscopic level. Quantitative parameters (cell number, cell shape and size, number and length of neurites) were derived with the use of image analysis (Quantimet 920).

The exposure of the septal cells to the NGF (100 ng/ml) resulted in a 2 to 3 fold increase in ChAT enzymatic activity. GM1 at a concentration of 50 ug/ml was found to increase the specific activity of ChAT less dramatically than NGF, but was found to enhance fiber outgrowth when compared to control cultures. Concentration of GM1 above 50 ug/ml proved to be cytotoxic. There appeared to be differential effects of these two factors which depended upon the cell seeding density of the septal cells in culture.

In conclusion, although NGF and GM1 were effective in increasing ChAT activity and neurite density it appears that they may act by different mechanisms.

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445.13 **EFFECTS OF INSULIN-LIKE GROWTH FACTOR II (IGF-II) ON HIPPOCAMPAL AND SEPTAL NEURONS MAINTAINED IN TISSUE CULTURE.**
S.M. Onifer, S.D. Farber, S.H. Murphy, Y. Kaseda, and W.C. Low, Dept. of Physiology and Biophysics, Indiana University School of Medicine, Indianapolis, IN 46223.

Receptors for insulin-like growth factor II (IGF-II) have recently been localized in pyramidal and granule cell layers of the hippocampal formation. Although the function of IGF-II in the central nervous system has yet to be elucidated, IGF-II has been shown to enhance neurite extension of sensory and sympathetic neurons in tissue culture. In order to determine whether IGF-II has similar actions on neurons in the hippocampal formation and septal nucleus, primary cultures from these areas of brain were maintained in media containing various concentrations of IGF-II.

Hippocampal and septal tissue were dissected from fetal rat pups 15-19 days in gestation and placed in oxygenated CEM 2000 media (Scott Lab). Cells were dissociated using 0.125% collagenase and 0.05% dispase in HAM's F-12. Suspensions were centrifuged and supernatants were resuspended in CEM 2000. Cells were then plated on to Falcon Primera flasks for five days to establish cell attachment. After the fifth day, fresh CEM 2000 media was introduced with 0, 0.1, 1.0, or 10 nM IGF-II.

Three days after the addition of IGF-II there appeared to be more phase-bright neuronal somata in cultures containing IGF-II in comparison to control flasks without IGF-II. Moreover, neurons in cultures containing IGF-II displayed neurite outgrowth with extensive branching patterns. Extensive neurite outgrowth and branching were observed for cultures of both hippocampal and septal neurons. Hippocampal and septal cultures without IGF-II, on the other hand, contained few neurons with neurite outgrowth, and the outgrowth from these neurons displayed no branching. These results suggest that IGF-II exerts a trophic effect on hippocampal and septal neurons of the central nervous system resulting in the extension and branching of neurites.

(This work was supported by a grant from Eli Lilly & Co.)

445.14 IGF-1 SUPPORTS SURVIVAL AND DIFFERENTIATION OF FETAL RAT BRAIN NEURONS IN SERUM-FREE HORMONE-FREE DEFINED MEDIUM. R. Fellows, A. Al-Hader* and R. Kadle*. (SPON: L. Marshall). Department of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242.

Dissociated fetal rat brain neurons can be maintained under serum-free conditions with insulin as the only trophic factor. In this study, we have investigated the effects of IGF-1 on the survival and differentiation of neurons cultured from fetal rat telencephalon and diencephalon-mesencephalon. Dissociated cells were obtained from 19-day fetal rat brains as described earlier (Ahmed et al., J. Neuroscience 3:2448, 1983). Cells were plated on poly-D-lysine coated plates or multi-well clusters in a defined medium containing insulin or IGF-1. The medium was replaced 20 minutes after plating and then every third day. Immunocytochemical staining for GFAP and neuron-specific enolase indicated that >95% of cells were neurons. Survival and morphological differentiation of neurons required insulin or IGF-1 and was dose dependent over the range of 1, 10 and 100 ng/ml IGF-1 or 10, 100 and 1000 ng/ml insulin. In general, the morphology of cells cultured with IGF-1 was comparable to that of cells grown in a ten fold higher concentration of insulin. Both insulin and IGF-1 increased dopamine content of diencephalic-mesencephalic cultures measured by HPLC with electrochemical detection. After three days in culture, dopamine content in the presence of 100 and 1000 ng/ml insulin was 102 and 126 percent of control respectively, while in the presence of 10 and 100 ng/ml IGF-1 it was 183 and 240 percent of control. These results indicate that IGF-1 at physiological concentrations supports the survival and differentiation of fetal rat brain neurons in serum-free hormone-free culture. In addition, our observations indicating an early predominance of IGF-1 receptors in fetal rat brain neuronal cultures suggest a role for IGF-1 in the in vivo development and differentiation of CNS neurons. Supported by NIH and Iowa DERC.

446.1 TARGET REGULATION OF NEUROTRANSMITTER PROPERTIES IN RAT SYMPATHETIC NEURONS: EVIDENCE FROM SWEAT GLAND TRANSPLANTATION.
R.J. Schotzinger* and S.C. Landis. Dept. of Pharmacology, Case Western Reserve Univ., Cleveland, OH 44106.

Most sympathetic neurons are adrenergic, however a minority are cholinergic. The best characterized cholinergic sympathetic neurons are those which innervate eccrine sweat glands in the footpad of the rat. Although the adult innervation is cholinergic, the innervation of the rat eccrine sweat gland initially displays catecholaminergic properties, such as catecholamine fluorescence and tyrosine hydroxylase (TH) immunoreactivity (IR). As development proceeds, these adrenergic traits are gradually lost, while the innervation begins to acquire choline acetyltransferase (ChAT) activity, acetylcholinesterase (AChE) activity and vasoactive intestinal polypeptide (VIP) immunoreactivity, properties of the adult cholinergic innervation.

To investigate the possible role of the target, the sweat gland, in this developmental plasticity, transplantation experiments have been performed in neonatal Lewis inbred rats. Hairy skin, in the lateral thorax region, was replaced with sweat gland-containing footpad skin. Hairy skin normally receives adrenergic sympathetic innervation, especially the piloerectors, but not cholinergic sympathetic innervation. The sweat gland-containing footpad skin normally receives predominantly cholinergic sympathetic innervation. This paradigm thus allows sympathetic neurons, which would normally innervate adrenergic targets in the hairy skin, to innervate sweat glands instead.

The innervation of the transplanted sweat glands was examined from 1 to 6 weeks post-transplantation (PT). Catecholamine fluorescent fibers first invaded the transplanted glands at 2 weeks PT, and formed an intensely fluorescent plexus around the glands by 3 weeks PT. In contrast, at 4,5 and 6 weeks PT only faintly fluorescent fibers were observed. The appearance and subsequent decrease in TH-IR followed the same time course. AChE was first seen at 3 weeks PT, became more intense at 4 weeks PT, and was maintained at 5 and 6 weeks PT. VIP-IR appeared in the innervation of many glands at 4 weeks PT, and became more intense at 5 and 6 weeks PT. Only occasional Substance P and Neuron Peptide Y fibers were evident at all timepoints examined. ChAT activity was assayed by synthesis of acetylcholine in the transplanted footpads. Low levels of ChAT activity, 7.4 pmol/mg prot/min, were present at 3 weeks PT, the first timepoint examined, and had increased to 33.2 pmol/mg prot/ min at 6 weeks PT.

These results suggest that the transplanted sweat glands are capable of regulating neurotransmitter phenotype in sympathetic neurons, in that target appropriate properties are observed in neurons that would not normally express these traits.
(Supported by NIH grant NS023678)

446.2 IN VIVO ENHANCEMENT OF A PARTIAL CATECHOLAMINERGIC PHENOTYPE IN CHOLINERGIC NEURONS OF THE MOUSE CILIARY GANGLION
P. C. Jackson Dept. of Anatomy, Dalhousie Medical School, Halifax, Nova Scotia

As far as is known all parasympathetic neurons in the mouse ciliary ganglion are functionally 'cholinergic' but curiously, many of these neurons also possess tyrosine hydroxylase (TH) detectable by immunofluorescence; they do not, however, produce catecholamines in vivo.

Axons of individual neurons in the ciliary ganglion (CG) and the sympathetic superior cervical ganglion (SCG) have collaterals both in constrictor and dilator muscles of the ipsilateral iris. Surgical removal of an SCG produces a complete sympathetic,but overall partial, denervation of the iris. We have observed at least two consequences of this manipulation: an intensification of fluorescence in ipsilateral TH-positive CG neurons, accompanied by an increase in the proportion that exhibit detectable TH-like immunofluorescence, and a modest but significant increase in the average polyneuronal innervation of CG neurons by their preganglionic inputs. There appears to be a denser than normal plexus of esterase positive axons in the irides innervated by these neurons, suggesting that parasympathetic neurons were induced to expand their axonal arborizations in response to sympathectomy. These effects are restricted to the sympathectomized side.

We adopted the working hypothesis that the observed influence of sympathectomy is mediated locally through the target tissue since a direct synaptic connection between the CG and the SCG defies demonstration. This led us to implant denervated iris tissue into the anterior chamber of the eye as a challenge to the host parasympathetic innervation and thus to determine firstly, whether axons were induced to grow into the implanted iris and secondly, if the cell bodies in the ipsilateral CG exhibited an enhanced TH-immunofluorescence. They did.

Our results to date consistently indicate that the response of parasympathetic neurons to an implanted iris parallels that produced by an ipsilateral sympathectomy. We are testing the hypothesis that an elevated level of Nerve Growth Factor in the vicinity of parasympathetic terminals is responsible.

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446.3 Cellular Localization of Nerve Growth Factor Synthesis by in situ Hybridization.
C. Bandtlow*, R. Heumann*, M.E. Schwab*[1], and H. Thoenen. Dept. of Neurochemistry, Max-Planck-Institute for Psychiatry, D-8033 Martinsried, F.R.G. and [1]Institute for Brain Research, Ch-8029 Zurich, Switzerland. (SPON: Y.A. Barde)

In both the peripheral and the central nervous systems a positive correlation exists between the levels of NGF and its $mRNA^{NGF}$ on the one hand and between these levels and the density of innervation by NGF responsive neurons on the other hand. By developing a sensitive and specific in situ hybridization technique we have been able to identify the cell types synthesizing $mRNA^{NGF}$. NGF is an extremely rare message, present at less than one copy per million molecules of $polyA^+$-RNA. It was therefore necessary to use probes with high specific activities, either ^{32}P- or ^{35}S-labelled. We chose ^{35}S-labelled probes because they give better resolution than is possible with ^{32}P. The disadvantage of ^{35}S is that it often results in high non-specific background, thereby precluding detection of rare messages such as $mRNA^{NGF}$. Thus, an improvement of available techniques was required. Prehybridization with unlabelled thio-UTP at pH 5.5 and the substitution of DTT with the more heat stable ß-mercaptoethanol were essential to decrease the serious background problems. With this improved method it was possible to localize $mRNA^{NGF}$ in tissues excusively innervated by sensory fibers (developing mouse whisker pad) and in tissues innervated by both sympathetic and sensory fibers (rat iris). The results indicated that $mRNA^{NGF}$ is synthesized by target cells of sensory and sympathetic fibers (i.e. epithelial cells, smooth muscle cells and fibroblasts) and not exclusively by Schwann cells ensheathing them. Moreover, in the newborn rat sciatic nerve (comprising sympathetic, sensory and motorfibers) and also in the distal part of transected nerves of the adult rat, all Schwann cells have the capacity to synthesize $mRNA^{NGF}$. In situ hybridization of primary cultures from various brain regions of prenatal rats (E17) showed specific labelling of subpopulation(s) of non-neuronal cells, having astrocyte-like morphology. Further characterization of these subpopulation(s) by immunohistochemistry and an evaluation of possible regional differences are in progress, as is an investigation of the mechanism of regulation of $mRNA^{NGF}$ synthesis.

446.4 REGULATION OF SYNTHESIS OF NERVE GROWTH FACTOR (NGF) AND ITS RECEPTOR.
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In situ hybridization experiments have demonstrated that in the target areas of sensory and sympathetic neurons NGF is produced by a great variety of cell types such as epithelial cells, smooth muscle cells, fibroblasts and also by Schwann cells ensheathing the axons of NGF-responsive neurons. It was also demonstrated that in adult animals (rats) the contribution of the Schwann cells to the total NGF supply is negligible. However, after lesioning of the sciatic nerve there was a rapid increase in the mRNA of NGF ($mRNA^{NGF}$) in the non-neuronal cells followed by re-expression of NGF receptors ($mRNA^{rec}$) on the Schwann cells. $mRNA^{rec}$ is normally expressed only during earlier developmental stages, suggesting that lesioning of the sciatic nerve results in a functional regression into earlier developmental stages. We have therefore investigated the developmental changes in the levels of $mRNA^{NGF}$ and $mRNA^{rec}$ and compared them with the changes occurring in adult animals after nerve lesioning. At birth the levels of $mRNA^{NGF}$ and $mRNA^{rec}$ in the rat sciatic nerve were 10 and 120 times higher respectively than in adult animals. $mRNA^{rec}$ levels steadily fell from birth approaching adult levels by the third postnatal week, whereas $mRNA^{NGF}$ levels fell only after the first postnatal week, reaching adult levels by the third week. Transection of the sciatic nerve resulted in a marked biphasic increase in $mRNA^{NGF}$ which was confined proximally to the area immediately adjacent to the cut; peripherally a marked elevation was present in all segments. $mRNA^{rec}$ levels were also markedly elevated after cut distally to the transection site in agreement with previous results obtained by immunological methods (Taniuchi et al., 1986, PNAS 83:4094-4098). Following a crush lesion (allowing regeneration) the $mRNA^{rec}$ levels were rapidly down-regulated with the arrival of regenerating nerve fibers. The down-regulation of $mRNA^{NGF}$ was slower and not as extensive as that of $mRNA^{NGF}$ receptor. When pieces of transected sciatic nerve were kept in culture, changes in the $mRNA^{rec}$ levels were similar to those in vivo; however for $mRNA^{NGF}$ only the initial rapid increase was observed. The in vivo situation could be mimicked only when activated macrophages were added to the cultures. This reflects the in vivo situation, where after nerve lesion macrophages immigrate into areas of Wallerian degeneration. Activated macrophages themselves do not synthesize detectable quantities of $mRNA^{NGF}$. The nature of these macrophage signals is under investigation.

446.5 STRUCTURAL FEATURES OF NERVE GROWTH FACTOR RECEPTORS FOUND ON NEURONAL VS. IMMUNOLOGIC CELL TYPES. C. Beck*, B. Morgan*, R. Stach, D. Marchetti*, L. Thorpe* and J.R. Perez-Polo. (SPON:B. Haber).Dept. of Human Biological Chemistry and Genetics., Univ. of Texas Medical Br. Galveston, Tx. 77550

The nerve growth factor protein, NGF, has been shown to play a central role in the regulation of cell death phenomena for some neurons of the peripheral nervous system and lesioned neurons of the basal forebrain. NGF may also function in an immunoregulatory capacity.

It has been demonstrated that the effects of NGF are mediated through binding and internalization of the beta NGF subunit by specific receptors on the surface of NGF responsive cells. We have compared structural and NGF binding properties of NGF receptors present in the rat pheochromocytoma PC12 and the human neuroblastoma LAN-1, both models for NGF responsive neurons, as well as human peripheral blood mononuclear cells (hPBMC) and rat splenic mononuclear cells (rMC). When equilibrium binding constants were determined for the PC12 and LAN-1 cells using a soluble receptor assay we found equilibrium binding constants of 10^{-9}M and 10^{-11}M, whereas for the mononuclear leukocytes we found only one equilibrium binding constant of 10^{-9}M. When proteins on the surface of cells were labelled with ^{125}I, cells solubilized, immunoprecipitated with monoclonal antibodies to the NGF receptor and analyzed by SDS-PAGE, we found the following:

Cell Type	NGFR(KDa)		
PC12 (rat)	207	-	63
LAN-1 (human)	200	92.5	-
rMC (rat)	178	119	-

Similar results were obtained for rat derived cells irrespective of whether we used MC192, a monoclonal antibody to rat NGF receptor, indirect immunoprecipitation with sequential exposure to NGF and then anti-NGF or an antiidiotypic antisera to the NGF receptor developed by us. These results would suggest that in spite of different effects of NGF on different tissues and striking antigenic differences between NGF receptors from rat vs human tissues there are strong structural similarities in the receptors present. Supported in part by NINCDS grant NS18708.

446.6 DEVELOPMENT OF THE CHICK TRIGEMINAL REGION: ^{125}I-NGF BINDING, HNK-1 AND NEUROFILAMENT IMMUNOREACTIVITY. D.B.Wayne* and M.B.Heaton. Dept. of Neuroscience, Univ. of Florida, Gainesville, FL 32610.

This study was a continuation of our investigations of the developing trigeminal (V) system in the chick embryo. Previous work in our lab has shown that in vivo, trigeminal motoneurons fail to extend their axons in the absence of the trigeminal ganglion. In vitro studies on the effects of ganglion conditioned medium on neural tube explants containing the V motoneuron population led to the observation that NGF was able to increase neurite outgrowth from these preparations.

Using ^{125}I-NGF we have examined NGF binding in the trigeminal region. We have seem binding in this region as early as embryonic day 4. There is binding in the ganglion and along the peripheral nerve. Heaviest binding is at the point where the sensory fibers enter and the motor fibers exit the brainstem, the equivalent of the dorsal root entry zone. There also appears to be labeling of the early motoneuron cell bodies once they accumulate in the lateral brainstem. The motor root courses through the medial aspect of the trigeminal ganglion and does not appear to heavily bind NGF as the sensory fibers do where they accumulate to enter the brainstem.

HNK-1 and neurofilament antibodies (provided by M.Bronner-Fraser and G.Bennett respectively) were used to more fully describe the disposition of fibers and cell bodies in the trigeminal region. Both HNK-1 and the neurofilament antibodies confirm that the entry zone which was so heavily labeled in the NGF binding study consists exclusively of accumulated fibers. The entry zone region of the brainstem can be identified at 2 days of incubation by the accumulation of migrating neural crest cells at this point. This is clearly seen with the HNK-1 antibody. The origination of the motoneurons in the medial cell column and their lateral translocation, as well as the presence of the motor root within the medial aspect of the trigeminal ganglion is clearly delineated with the neurofilament antibody.

Currently, the observations on NGF binding in this region are being extended to include earlier time points. (Supported by NIH grant NS-20387 to M.B.H.)

446.7 NERVE GROWTH FACTOR RECEPTOR-MEDIATED TRANSPORT FROM CEREBROSPINAL FLUID TO BASAL FOREBRAIN NEURONS. John B. Schweitzer, Dept. of Pathology, Univ. of Tennessee, Memphis, TN 38163.

Recent data indicate that the neurons of the cholinergic basal forebrain (CBF) respond to nerve growth factor (NGF) with increased survival under experimental conditions and have NGF receptors which mediate the binding and retrograde transport of NGF from axon terminals to somata. The distribution of monoclonal antibody 192 (Mab 192), a well-characterized antibody which recognizes only the rat NGF receptor, was investigated following intraventricular injection. Mab 192 was iodinated using standard techniques; a rat monoclonal antibody directed against mouse immunoglobulin, Mab 187, was iodinated and used as a control. Two rats in each of four experimental groups received intraventricular injections: 1) 10 pmoles (1.5 ug) of ^{125}I-192; 2) 3 pmoles (0.45 ug) of ^{125}I-192; 3) 0.45 ug of ^{125}I-192 plus 43 ug of unlabelled Mab 192; and 4) 5 pmoles (0.8 ug) of ^{125}I-Mab 187. Twenty-four hours after intraventricular injection, the animals were anesthetized and perfused with 4% paraformaldehyde. The brains were removed and embedded in paraffin. The brain was serially cut and sections obtained at intervals and processed for autoradiography. Highly specific bilateral transport of ^{125}I-Mab 192 to numerous neurons of the CBF system following either dose of ^{125}I-Mab 192 was demonstrated by autoradiography. Specific labelling of discrete regions of neuropil consistent with terminal fields was also observed. Aggregates of silver grains around the outer aspects of leptomeningeal vessels were present and can be ascribed to the presence of sympathetic terminals; ^{125}I-Mab 192 was also prominently retained around many intraparenchymal vessels. The labelling of neuronal somata, discrete regions of neuropil, and vessels was virtually abolished by co-administration of a 95 fold greater concentration of unlabelled Mab 192. Labelling of neurons, neuropil or vessels did not occur with ^{125}I-Mab 187. Since retrograde tracing studies demonstrate that the distribution of axons from cholinergic nuclei to cortex and hippocampus is topographically restricted and largely ipsilateral, dissemination of the labelled Mab 192 in the CSF pathways with subsequent diffusion into neuropil and uptake by NGF receptors would best explain the multiplicity and bilaterality of the labelled cholinergic nuclei. This result directly demonstrates that suitably targeted antibodies can be taken up by specific neuronal populations following intraventricular injection and implies that CBF neurons may be influenced by relatively high molecular weight substances injected into cerebrospinal fluid. This work was supported by NSF grant BNS-8603593.

446.8 A BALANCE OF BETA-ENDORPHIN/INSULIN LEVELS APPEARS NECESSARY FOR THE NORMAL DEVELOPMENT OF THE BRAIN. J.V. Bartolome*, M.B. Bartolome*, E.B. Harris* and S.M. Schanberg* (SPON.: E.Hallman). Department of Pharmacology, Duke University Medical Center, Durham, N.C. 27710

A considerable body of evidence now exists indicating that opioid peptides may have an important role in the regulation of brain growth and development. Our laboratory has recently shown that intracisternal (i.c.) administration of beta-endorphin (BE) to neonatal rats markedly decreases brain ornithine decarboxylase (ODC) activity. ODC catalyzes the intial step in the synthesis of the polyamines spermidine and spermine which are major regulators of cell growth, multiplication and differentiation. Previous studies have shown that changes in ODC levels are invariably associated with altered tissue maturation. Therefore, our data suggest that endogenous BE functions to down-regulate brain cell growth during perinatal development.

While it is well established that insulin influences the cellular development of many organs, little is understood of its role on brain cell maturation. Recent observations suggest that insulin or insulin-like peptides acting alone or in concert with "other factors" may play a major role throughout brain growth. Our study examines the effect of insulin on brain ODC and whether tissue ODC responsiveness to this trophic hormone is affected by changes in the levels of BE in the CNS.

Subcutaneous (s.c.) administration of insulin (20 U/kg) to 6-day-old rats produced a small but significant decrease in brain ODC activity. In agreement with our previous studies, a substantial decrease in ODC activity was also seen in rat pups given 1 µg of BE i.c. However, surprisingly, brain ODC was markedly stimulated in 4- and 6-day-old animals given BE i.c. plus insulin s.c. This action of BE appears to be restricted to early development as the peptide did not affect brain ODC responses to insulin in weaned animals. Intracisternal administration or morphine or naloxone did not affect brain ODC responses to insulin.

These data suggest that (1) insulin can regulate brain growth through actions on ODC, (2) this action of insulin on brain development is influenced significantly by BE; depending on the concentration of BE in the CNS the effect of insulin on brain growth can be inhibitory (basal CNS BE level) or stimulatory (supranormal CNS BE level), and (3) the modulatory action of BE on brain ODC responsiveness to insulin is not mediated by classical opioid receptors.

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446.9 ANALYSIS OF ABNORMAL CORTICAL MORPHOGENETIC EVENTS INDUCED BY CHOLINERGIC BASAL FOREBRAIN LESIONS AT BIRTH. C.F. Höhmann, G. Forloni, M.L. Oster-Granite and J.T. Coyle. Depts. of Neurosci. and Physiology, Johns Hopkins Univ. Sch. of Med., Balto. MD 21205.

We previously reported that destruction of cholinergic afferents from basal forebrain nuclei induces abnormal cortical morphogenesis in the Balb/c mouse. Unilateral electrolytic lesions were placed, at birth, in the magnocellular basal forebrain nuclei [nBM] which project to fronto parietal cortex. A severe disruption of cortical cytoarchitecture resulted, ipsilateral to the lesion, in all areas that displayed reductions of acetylcholinesterase [AChE] histochemistry. Lesions of noradrenergic, serotonergic or thalamocortical afferents did not produce comparable developmental abnormalities.

The lesion-induced abnormal cytoarchitectonic pattern most likely resulted from a delay in neuronal maturation. Cells in several cortical layers retained an undifferentiated appearance, ipsilateral to the lesion, much longer than in the contralateral hemisphere, but consecutively assumed a more differentiated appearance concurrent with the recovery of cholinergic markers. In the adult the most prominent abnormality was an absence of a distinctive cortical layering ipsilateral to the lesion. The present experiments examine whether our hypothesis of a lesion-induced delay in neuronal maturation is correct or whether other events, such as abnormal cortical cell migration or neuronal loss in cortex, may occur. To this end we have monitored the fate of neurons labelled by 3H-thymidine [3H-Thy] at the time of their generation. Further, we assesed the neurochemical development of GABAergic and somatostatin [SOM] neurons intrinsic to frontoparietal cortex.

Time-mated dams were injected i.p. with three daily doses of 50μCi of 3H-Thy on gestational days [E]16,17 and 18. Their pups received unilateral nBM lesions at birth, were formalin perfused on PD5 or PD30, sectioned frozen at 50μ and processed for autoradiography. Glutamic acid decarboxylase [GAD] enzyme activity was measured to assess GABA synthesis in cortical neurons. Further, SOM neurons were monitored in cortex immunohistochemically. Neurons labelled by 3H-Thy incorporation on E16,17 or 18 were found in the same location (layers II,III and to some small extent IV) in ipsilateral to lesion and contralateral cortex. The amounts of labelled cells in the two hemispheres were also comparable. Thus, cell migration apparently proceeds normally despite the lesion and cell numbers at least in layers III and II are not reduced. No difference in GAD activity and, intrinsic to cortex, SOM-IR cells could be found between ipsilateral to lesion and contralateral or control cortices, further indicating that neuronal death is an unlikely cause for the cortical maldevelopment. However, a transient population of SOM-IR cells appeared ipsilaterally, in the sub-cortical white matter, 1-3 weeks following the lesion. We are now exploring the relationship between these cells and "Cajal-Retzius" type neurons found in similar locations in early development.

The present results are compatible with the hypothesis of maturational delay as the consequence of cortical cholinergic deafferentiation at birth. In addition, the absence of differences in cortical GAD activity suggests that the neurochemical maturation of cortical cells is not impaired to the same degree as their morphological ontogeny and thus is probably determined prior to the lesion. *Supported by PO1 HD19920 and the McKnight Foundation.*

446.10 IMMUNOCYTOCHEMISTRY AND IN SITU HYBRIDIZATION STUDY OF SOMATOSTATIN EXPRESSION IN MOUSE BRAIN DURING NORMAL DEVELOPMENT AND AFTER NEONATAL LESION OF BASAL FOREBRAIN. G. Forloni, C.F. Höhmann, C. Bendotti, B. O'Hara*, R. Reeves*, J.D. Gearhart*, M.L. Oster-Granite and J.T. Coyle. Depts. of Neurosci. and Physiol., Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

An interaction between somatostatin (SOM) neurons and cholinergic projections to the cortex has been suggested by the concomitant reduction in presynaptic cholinergic markers and SOM levels in Alzheimer's disease. Since plastic interactions between neuronal systems may be amplified in the developing brain, we examined the effects of lesions of the basal forebrain cholinergic neurons of the newborn mouse on the subsequent development of cortical SOM neurons. These lesions have been shown to cause a disruption of normal morphogenesis in neocortex (see preceding abstract).

BALB/C mice received unilateral electrolytic lesion in the ventral globus pallidus magnocellular region [nBM] within 24 hours of birth. Animals were killed on postnatal days (PD) 3,5,7,10,14,21 and 30 by transcardial perfusion with buffered 4% paraformaldehyde. Coronal sections were processed for SOM immunocytochemistry with two different polyclonal antisera against SOM-14 and SOM-28 and a monoclonal antibody against SOM-14. In adjacent sections, SOM mRNA was visualized by *in situ* hybridization with a rat preprosomatostatin [^{35}S]cDNA probe. Finally, adjacent sections were processed for acetylcholinesterase (AChE) and NADPH diaphorase histochemistry, a putative enzymatic marker for SOM neurons.

Distribution of SOM immunoreactivity (SOM-IR) in the mouse forebrain was similar to that reported for rat. Regions with substantial numbers of SOM-IR neurons include hypothalamus, hippocampus, striatum, amygdala and neocortex. The distribution of SOM-IR and SOM gene expression corresponded with high fidelity in all regions examined from PD 3 to 30.

As reported in the preceding abstract, distribution and numbers of somatostatin immunoreactive cells were not markedly changed within cortical regions displaying AChE depletion and cytoarchitectonic abnormalities. However, we observed a transient increase in the number of SOM-IR neurons in the alveolar and oriens layer of the hippocampus, ipsilateral to the lesion, between PN 7 and 21. During the same period, transient, SOM-IR cells were observed, ipsilaterally, in the white matter near the superior edge of the corpus callosum at the level of the frontal cortex. The *in situ* hybridization studies confirmed an aberrant distribution of neurons expressing SOM in these regions. In contrast, no change was observed in the distribution of NADPH diaphorase-positive cells in the hippocampus in response to the lesion and their distribution did not correspond with SOM-IR.

The present study shows a close temporal and spatial correspondence in the appearance of SOM-IR neurons and the gene expression of SOM in the telencephalon of the mouse. Furthermore, our results suggest that the impact of nBM lesions on the development of neuronal populations is even more extensive than our previous results indicated. *Supported by: PO1 HD19920, RO1 HD19932 and the McKnight Foundation.*

446.11 DIFFERENT MORPHOLOGIC CHANGES ARE PRODUCED IN THE DORSAL COLUMN NUCLEI OF ADULT RATS FOLLOWING ANTEROGRADE, RETROGRADE, OR COMBINED ANTEROGRADE-RETROGRADE TRANSNEURONAL DEGENERATION. Mary Lou Voytko[1,2] and Dennis J. Stelzner[2]. [1]Lab. of Neuropathology, Johns Hopkins Sch. of Med., Baltimore, MD 21205 and [2]Dept. of Anatomy and Cell Biology, SUNY Hlth. Sci. Ctr. Syracuse, Syracuse, NY 13210.

Transneuronal degeneration has been observed in both the CNS and PNS. The anterograde phenomenon has generally been dealt with more extensively than the retrograde. The interaction of these effects on the same neurons has been overlooked for the most part. Therefore, the present study focused on morphologic changes induced by anterograde (deafferentation) and/or retrograde (target removal) transneuronal degeneration in the dorsal column nuclei (DCN) of adult rats. These nuclei are ideal for this study since these alterations can be examined without the confounding influence of direct damage to the neurons themselves. This study focused on the dorsocaudal thalamic projecting cell population within the DCN.

A bilateral midthoracic dorsal funiculotomy and a unilateral sensorimotor cortex lesion were performed in a one-stage operation in 3 mos. old rats (N=4). This combined surgery used all four DCN to provide all the experimental [anterograde (ANT) and retrograde (RETRO) alone, and combined anterograde-retrograde (AR)] and control conditions in each animal. The animals survived 60 days post-operatively, were perfused and histologically prepared. Unoperated control animals (N=4) were similarly prepared. Measurements of somal area, area of the DCN itself, and cell counts were made in each of the 4 nuclei in all animals.

The control nucleus within each experimental animal was not significantly different from this same nucleus in unoperated controls on any of the parameters measured. Anterograde effects alone did not influence cell number, but greatly decreased somal area and to a lesser extent area of the DCN itself. Retrograde changes involved a significant reduction of both cell number and area of the DCN. The AR interaction surprisingly had no greater effect on cell number than RETRO and was not significantly different from controls. There was a strong but nonsignificant influence on somal area that was similar to that seen with ANT. The greatest AR effect was seen in the area of the DCN; it was smaller than in ANT or RETRO.

The results indicate that within the DCN 1) the AR interaction produces changes that are mainly additive rather than synergistic and 2) ANT or RETRO transneuronal degeneration may work by different mechanisms since they appear to differentially influence neuronal parameters. (Supported by NINCDS NS07777 and NS14096.)

446.12 COMBINED EFFECTS OF GM1 GANGLIOSIDES AND FETAL BRAIN TISSUE TRANSPLANTS ON RECOVERY FOLLOWING SENSORIMOTOR CORTEX LESIONS. M.D. Slavin,* S. Lesensky, E. Curran,* A.M. Gentile and D.G. Stein. Brain Res. Lab., Clark University, Worcester, MA 01610 & Motor Learning Lab. Teacher's Coll., Columbia Univ., New York, NY 10027.

Adult male rats were first trained to shuttle back and forth on an elevated runway to obtain water reward (Gentile, A.M. et al, Behav. Biol. 22:417, 1978). Once this training was complete and criterion scores determined, the rats were given either sham operations (no brain tissue removed, n=8) or bilateral, single stage ablations of the sensorimotor cortex. Two groups of rats (n=8 & 9 respectively) received 30mg/kg i.p. injections of GM1 gangliosides for 14 days beginning with the day of surgery, while the remaining animals received equivalent volumes of saline. After 7 days, one group with GM1 injections received bilateral implants of E18-19 fetal frontal cortex directly into the wound area. One group of rats that received saline injections was also given similar brain tissue grafts. To assess recovery, animals were tested on the runway task 7 days later. During the initial 8 day testing period, all groups except the one receiving transplants plus GM1 had significantly longer running times than the sham operated group ($p < 0.05$). This group also had significantly fewer foot-fault errors than rats with lesions alone during the last 4 days of testing. To determine the long-term effect of treatment, animals were retested 42 days after reaching their criterion scores. Retesting revealed that the group receiving GM1 alone had running times that were significantly longer than both the sham group and those with transplants plus GM1. The transplant plus GM1 and shams also had fewer foot-faults than the other experiental groups. To provide closer inspection of the process of recovery, the rats were filmed the day after they reached criterion performance and subsequently again after a 42 day interval. Initial analysis of movement revealed that all lesion groups were significantly different from shams. We conclude that neither GM1 nor transplants alone enhanced recovery; however with combined treatments the animals achieved time and error scores that were not significantly different from unoperated controls. (Supported by American Paralysis Association and Fidia Research Laboratories).

446.13 **LOCAL AND SYSTEMIC EFFECTS OF MUSCLE REGENERATION ON MOTOR AXON REINNERVATION.** K.E. Misulis, M.E. Clinton*, and W-D. Dettbarn. Neurology & Pharmacology Depts. Vanderbilt Univ., Nashville, TN 37212

Previous studies from our laboratory have shown that motor axon reinnervation is accelerated when one hindlimb muscle is injected with bupivacaine. This causes necrosis with subsequent regeneration of the muscle. We found that not only did the injected muscle have accelerated reinnervation, but adjacent noninjected muscles had acceleration of reinnervation, as well.

The first part of this study examined whether the acceleration of reinnervation of muscles adjacent to those injected with bupivacaine was due to spread of local inflammation. Histologic sections were made of the soleus and extensor digitorum longus (EDL) at 1 to 28 days after injection of the soleus with bupivacaine. Whereas the injected soleus showed prominent inflammation and necrosis which was maximal at 2 days, there were only regions of mild inflammation in the adjacent EDL. No necrosis was seen.

The mild inflammation in adjacent muscles with bupivacaine injection could be responsible for the acceleration of reinnervation. Therefore, another method of producing necrosis was used. Either the soleus or EDL was maximally crushed using a small hemostat. This resulted in necrosis of only the operated muscle. With this treatment, there was still the acceleration of reinnervation of the damaged muscle, as measured electrophysiologically, but changes in the nonoperated muscle were not significant.

To more definitively determine whether there was a systemic effect of regenerating muscle, either the soleus or EDL was injected contralateral to the side of the nerve crush. Thus, this tested whether necrosis and regeneration on one side would influence reinnervation on the opposite side. These data showed no significant acceleration of reinnervation by the contralateral regeneration.

These data suggest that the acceleration of reinnervation with regeneration is due to local and not systemic effects, and that it is not a specific drug effect of bupivacaine. Also, these data raise the possibility that inflammation without necrosis may promote reinnervation.

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446.14 THE MECHANISM OF THE TROPHIC ACTION OF GABA. Paul Madtes Jr. and Geoffrey Fuller. Depts. Biology and Chemistry, Point Loma Nazarene College, San Diego, CA 92106.

Classically, γ-aminobutyric acid (GABA) acts as a major inhibitory neurotransmitter in the mature central nervous system (CNS). Recent evidence indicates that GABA plays a role in the development of its synaptic contacts. We have studied the relationships between the various biochemical components of the GABA system, using the rabbit retina as our model. If the activity of the uptake system, which removes endogenous GABA from the synaptic cleft, is blocked during development by either an *in vivo* or *in vitro* treatment with nipecotic acid, a GABA analogue; an increase in the number of GABA receptors results. This "up regulation," or "induction," of receptors only occurs in immature tissue since treatment of mature tissue results in a loss of receptors. This sensitivity to nipecotic acid is maximal around the time of eye opening and is light-dependent. While this induction has been shown to involve the GABA receptor, since treatment with GABA agonists mimics this effect; the mechanism by which it acts is unclear. This question has been addressed in this study. Newborn rabbit pups were delivered to the laboratory one day after birth and sacrificed. Eyecups then were prepared and incubated in the presence of various GABA analogues in order to determine the specificity of action. Co-incubation of nipecotic acid and THIP (a GABA agonist) resulted in an increase in binding equal to that obtained after treatment with either alone. This non-additive effect suggests that both act through the GABA receptor. Similar treatments did not increase low-affinity binding. In addition, the time required for the effect to appear was studied by incubating the tissue with nipecotic acid for various time periods. The level of binding was measured by ^{3}H-muscimol ligand binding to prepared membranes. An increase in high-affinity binding was observed as early as 25 minutes after treatment with nipecotic acid began. This suggests that the number of GABA receptors increases as a result of exposure of pre-existing receptors rather than *de novo* synthesis. Thus, blocking uptake by nipecotic acid probably raises synaptic levels of GABA, increasing the time of interaction of GABA with its high-affinity receptors. This would result in the rapid exposure of high-affinity receptors on the surface of immature membranes. These results further support the suggestion that GABA acts as a trophic factor in the developing CNS. Funded by a grant from Research Corp.

448 SYMPOSIUM. NEW INSIGHTS INTO THE STRUCTURE AND FUNCTION OF RECEPTORS. Z.W. Hall, Univ. of California, San Francisco (Chairperson); R. Dixon*, Merck, Sharp and Dohme Res. Labs; H. Bourne*, Univ. of California, San Francisco; C. Zucker, Univ. of California, San Diego; E.M. Shooter, Stanford Univ.

Molecular biology provides powerful tools for investigating the structure and function of proteins important for signaling in the nervous system. Experiments using these techniques have been particularly useful for detecting relationships between proteins, for defining their functional domains, and for investigating their cellular roles. The symposium will focus on recent progress in these areas specially related to the β-adrenergic receptor and the related protein, rhodopsin, to the family of G-proteins, and to the receptor for nerve growth factor (NGF).

449 **SYMPOSIUM. HORMONAL ORGANIZATION AND REORGANIZATION OF NEURAL CIRCUITS.** A.P. Arnold, UCLA (Chairperson); S.M. Breedlove, University of California, Berkeley; C.L. Jordan, UCLA; D.R. Sengelaub, Indiana University; J.W. Truman, University of Washington; R.B. Levine, University of Arizona.

Hormones can influence some of the major cellular processes responsible for the initial formation of some neural circuits. By modulating fundamental processes such as cell death, neuronal specification, synaptogenesis, synapse elimination, and morphogenesis of the dendritic tree, it appears that hormones determine not only the number of neurons devoted to specific tasks, but the pattern of their synaptic connections and their functional roles. Recent evidence suggests that some of these formative processes are not only influenced early in development, but may also be regulated rather dramatically at later stages of life as hormones reorganize neural circuits. Because these generalizations come from studies on a variety of hormone-sensitive vertebrate and invertebrate neural systems, it seems that they represent fundamental principles of hormone action on neurons.

The symposium is intended to explore two hormone-sensitive neural systems in some depth. These are the sexually dimorphic perineal neuromuscular system in rats (including the lumbar spinal nucleus of the bulbocavernosus), and the nervous system of the hawkmoth, *Manduca sexta*. Hormones control very different functions in these two systems, but there is surprising similarity in some of the cellular mechanisms of hormone action.

450.1 ESTRADIOL INCREASES SPONTANEOUS ACTIVITY OF HIPPOCAMPAL NEURONS IN CULTURE. J.H. Meyer and R.Robbins. Depts. of Internal Medicine, and Obstetrics and Gynecology. Yale University School of Medicine, New Haven, CT. 06510.

Steroid influences upon neuronal function have classically been though to result from genomic effects, which appear at long latencies and last for extended periods following administration of a steroid. There is increasing evidence, however, that steroids also exert non-genomic effects upon neuronal activity. Such effects may exist in the hippocampus, where estradiol 17 B (E_2) causes short-latency increases in CA1 field potential amplitudes (Teyler et al. Science 209: 1017). In a preliminary effort to understand the mechanisms of E_2's effect upon the hippocampus, we assessed the influence of E_2 upon the activity of hippocampal neurons in dissociated cell cultures from newborn rats.

Recordings were made from 2 to 5 week old cultures in a static bath containing 2 ml of a phosphate buffered saline solution with 1% gelded (estrogen-free) horse serum. A modified cell-attached patch clamp technique was used to record spontaneous action potentials from individual cells. Mean discharge frequencies were obtained over a 5 minute initial period, after which 10 µl of a test solution was added to the saline bath. 3 test solutions were used: phosphate buffered saline (PBS; control group), and a solution of ethanol in PBS with or without E_2 (E_2 and ethanol groups, respectively: EtOH test solution concentration=.02%, final saline bath concentration =.0001%; E_2 test solution concentration= 7.2×10^{-6}M, final saline bath concentration=3.6×10^{-8}M). Discharge frequencies over the subsequent 10 minute experimental period were obtained and the mean frequency over this period normalized to the mean frequency over the initial period (=normalized frequency).

Both steadily-discharging and bursting cells were encountered. For all cells (n=70), the mean frequency over the initial period was 0.93±.05 Hz (s.e.m.). In the control group, firing frequencies during the experimental period showed no apparent changes from the initial period (mean normalized frequency (MNF)=1.03±.13; n=28). A similar finding was noted among the ethanol group (MNF=1.00±.17; n=22). In the E_2 group, however, spontaneous activity displayed increases (MNF=1.64±.25; n=20) which were significantly different from the changes seen in the control and ethanol groups ($p<.05$; T-test). The maximal increase in frequency was noted 3 minutes following E_2 application, with lesser increases over the remainder of the experimental period.

The specificity of estradiol 17B's effect upon hippocampal cells remains to be determined, as does the mechanism of estradiol's action. Such studies will be important to an understanding of increased seizure activity related to high estrogen states.

Supported by NIH grants HD13587 and NS06208.

450.2 ESTRADIOL ALTERS THE RHYTHM OF β_1- AND β_2-ADRENERGIC RECEPTORS IN DISCRETE BRAIN AREAS OF OVARIECTOMIZED RATS. N.G. Weiland and P.M. Wise. Department of Physiology, School of Medicine, University of Maryland, Baltimore, MD 21201.

Previous studies have demonstrated that: (1) estradiol (E_2) alters the pattern of release of norepinephrine (NE) and LH in ovariectomized (OVX) rats; (2) NE can inversely regulate the density of its own receptors; and (3) in male rats, β-adrenergic receptors exhibit a rhythm in frontal cortex. Therefore, we wished to determine whether β_1 and β_2 receptor densities exhibit a diurnal rhythm in OVX rats and whether E_2 treatment alters this rhythm in discrete brain regions that control reproductive function.

Using quantitative autoradiographic methods, we measured the density of β_1 and β_2 adrenergic receptors in OVX and E_2 treated female rats. Rats were OVX and one week later they received Silastic capsules containing E_2. Two days later rats were killed at 0300, 1000, 1300, 1500, 1800, and 2300 h. Brains were removed and frozen. 20µ sections were sliced in a cryostat and mounted on gelatin-coated slides. Frozen sections were incubated with ^{125}I-pindolol in Tris-HCl buffer and either ICI-118,551 to measure β_1 receptors or ICI-89,406 to measure β_2 receptors. Non-specific binding was determined by the addition of excess isoproterenol. Dried slides were exposed to LKB Ultrofilm, and the films were quantitated using a computerized image analysis system (Image Tech. Corp.).

In OVX rats, a diurnal rhythm in β_1 receptor densities was detected in the median eminence, ventral medial nucleus, and the pineal gland, and a rhythm in β_2 receptors was observed in the suprachiasmatic nucleus. Estradiol treatment suppressed the rhythm of both β receptor subtypes in these areas. In addition, E_2 caused an overall suppression of β_2 receptor densities in the pineal gland. In summary, these data demonstrate that E_2 treatment eliminates the rhythm in β receptors, and therefore, this effect does not correlate with the pattern of NE turnover or LH secretion which is observed following E_2 exposure.

(Supported by NIH grants AG-02224, AG-00168, HD-15955, and AG-05357.)

450.3 **EFFECTS OF ULTRAVIOLET RADIATION AND VISIBLE LIGHT ON PINEAL MELATONIN IN DJUNGARIAN HAMSTERS.** G.C. Brainard, M.D. Rollag* and M.H. Stetson*. Department of Neurology, Jefferson Medical College, Philadelphia, PA 19107; Department of Anatomy, Uniformed Services University of Health Sciences, Bethesda, MD 20814; Physiology Section, School of Life and Health Sciences, Univ. of Delaware, Newark, DE 19716.

The Djungarian hamster, Phodopus sungorus, exhibits a nocturnal rhythm of pineal melatonin content. Exposure of this hamster to a one minute pulse of white light (more than 10^{16} photons/cm^2) at night rapidly suppresses the activity of the melatonin forming enzyme serotonin-N-acetyltransferase. The purpose of the following study was to test the capacity of equal photon densities of three different monochromatic wavelengths for suppression of nocturnal pineal melatonin content. One month old male Djungarian hamsters were maintained on a L:D 16:8 for 4 weeks prior to the experiment. Hamsters (N=7) were individually exposed to a monochromatic wavelength for 5 minutes and returned to darkness for 15 minutes before sacrifice. Matched control animals were handled in a similar fashion but not exposed to a monochromatic stimulus. After sacrifice, pineal glands were immediately removed, frozen at -20°C and later assayed for melatonin content by RIA. Three different wavelengths (360, 500 and 640 nm) were tested for their capacity to suppress pineal melatonin. Each exposure was balanced to 1.36×10^{15} photons/cm^2. The half-peak bandwidth for each wavelength was 10 nm. To produce these monochromatic wavelengths, broadband white light was collimated by condensing lenses, and filtered with glass neutral density, interference and infrared filters. Exposure to a 5 minute pulse of 1.36×10^{15} photons/cm^2 of 500 nm light caused a significant ($P<0.01$) reduction of pineal melatonin relative to matched unexposed control animals. In contrast, animals exposed to 1.36×10^{15} photons/cm^2 of 360 nm or 640 nm did not exhibit significant decreases in pineal melatonin relative to matched unexposed control animals. These data indicate that 500 nm light has a greater capacity for influencing pineal melatonin of Djungarian hamsters than either 640 nm or 360 nm radiation. Supported by Pfeiffer Foundation, USUHS Protocol C607049 and R07039 and NSF Grant #DCB 8412587.

450.4 INHIBITION OF N-METHYLNITROSOUREA-INDUCED MAMMARY CARCINOGENESIS BY MELATONIN. D.B.Pelletier*, S.M.Hill*, and D.E.Blask. Department of Anatomy, Univ. of Arizona Coll. of Med., Tucson, AZ 85724.

Recently, research on the effects of the pineal hormone melatonin (MEL) on hormone-responsive carcinogenesis, particularly breast cancer, has intensified. We have studied the effects of MEL on N-methylnitrosourea (NMU)-induced mammary carcinogenesis, a relatively new model of human breast cancer which is both estrogen (E_2) and prolactin (PRL) responsive. Our study was designed to test the following three hypotheses: 1) MEL inhibits mammary cancer growth, 2) Elimination of the endogenous MEL signal, via pinealectomy, promotes mammary cancer growth, and 3) MEL alters mammary cancer growth by altering the neuroendocrine regulation of pituitary PRL and/or ovarian E_2 secretion. Adult female Sprague-Dawley rats received intravenous injections of NMU (5 mg/100 gms body weight) at 50 and 57 days of age. Animals were divided into three experimental groups: 1) Pinealectomized (PINX)- the pineal gland was surgically removed from each animal. 2) MEL-treated- animals received daily injections of MEL (500 ug) in the late afternoon (1600 hrs). 3) Saline (SAL)-treated- control animals received daily injections of a SAL vehicle in the late afternoon. By the end of 19 weeks, 86% of the SAL animals had developed palpable tumors. However, only 67% of the MEL rats exhibited tumors, whereas tumor formation occured in 100% of the PINX animals. The total number of tumors in the PINX and SAL groups was essentially the same (45 and 42, respectively), whereas the MEL group developed only 13 total tumors. Also, the number of tumors per tumor bearing animal and the mean rate of tumor growth for the MEL group were significantly lower than control values. Serum levels of immunoreactive E_2 were not statistically different among the treatment groups. However, serum PRL levels were significantly higher in the PINX group when compared to controls. In conclusion, the data support the hypothesis that MEL inhibits and pinealectomy promotes NMU-induced mammary carcinogenesis. Although the mechanism of tumor inhibition is unclear, it may involve an interaction of MEL with E_2 and/or PRL directly at the level of the mammary tumor tissue rather than a neuroendocrine suppression of hormone secretion. [Supported in part by PHS Grant No. 1R01CA42424-01 from the National Cancer Institute.]

450.5 MELATONIN INCREASES SERUM INSULIN-LIKE GROWTH FACTOR-I (IGF-I) IN MALE SYRIAN HAMSTERS. J. Vriend*, M. S. Sheppard* and R. M. Bala* (SPON: K. Dakshinamurti). Dept. of Anatomy, Univ. of Manitoba Sch. of Med., Winnipeg, MB, Canada R3E 0W3 and Dept. of Medicine, Univ. of Sask., Saskatoon, Sask, Canada S7N 0W0.

Daily melatonin injections produce a syndrome in male Syrian hamster which includes gonadal atrophy, reduced levels of circulating thyroid hormones, and an increase in body weight. The objective of this study was to determine the effects of daily melatonin injections on circulating levels of the growth factor, IGF-I, in male Syrian hamsters. Melatonin was administered subcutaneously (25 micrograms in 0.1 ml saline) every day for six weeks to normal mature male hamsters (N=8) maintained on a 14L/10D photoperiod. Melatonin was also administered to hamsters receiving 5 micrograms thyroxine subcutaneously (in 0.1 ml saline) every other day. Additional control groups of hamsters received either daily injections of saline or injections of thyroxine every other day. All injections were administered 1 - 2 hrs before lights out. Body weights of the hamsters were taken weekly. At the end of 6 weeks the animals were weighed and decapitated and serum was collected for determination of IGF-I levels by radioimmunoassay (RIA). The displacement curve for hamster serum (acidified to 1% formic acid) was parallel to that of the purified human IGF-I standard. Serum levels of thyroxine were also determined by RIA. Weights of the testes were recorded.

At the end of 6 weeks gonadal weights were reduced to approximately 1/4 of controls and body weights were increased 10 grams above controls by melatonin or by T4 injections alone. Melatonin injections in combination with T4 injections resulted in an average body weight increase of 28 grams greater than controls ($p < .01$). A highly significant ($p < .01$) increase in serum IGF-I levels was obtained in serum of hamsters treated with melatonin alone (180 ± 13% of controls) or in combination with thyroxine (188 ± 15% of controls). Two-way analysis of variance showed that melatonin and T4 treatments had independent effects on body weights ($p < .01$), but that only melatonin treatments significantly elevated IGF-I levels ($p < .001$).

These data suggest that melatonin influences body weight via an IGF-I dependent mechanism that differs from the mechanism by which thyroid hormones influence body weight. The melatonin induced increase in IGF-I could be due to changes in growth hormone secretion. This explanation is consistent with a CNS site of action of melatonin. An alternative explanation is that melatonin acts directly on IGF-I secreting cells. (Supported by the Medical Research Council of Canada.)

450.6 LOW LEVELS OF CORTICOSTERONE REVERSE ADRENALECTOMY-INDUCED IMMUNOHISTOCHEMICAL STAINING OF CRF AND VASOPRESSIN IN THE PARAVENTRICULAR NUCLEUS OF ADRENALECTOMIZED RAT. Susan F. Akana and Mary F. Dallman*. Dept. Physiology, UCSF, San Francisco, CA 94143 USA.

We have shown the glucocorticoid sensitivity of a variety of physiological endpoints in adrenalectomized rats administered low levels of corticosterone (B) (Rec. Prog. Hormone Res. 43:113, 1987). The IC 50 for ACTH approximates 0.5nM whereas the IC 50 for thymus and corticosteroid binding globulin approximates 5nM. We speculated that regulation of ACTH secretion is by a type I, B-preferring receptor whereas the other two endpoints are regulated by a type II, glucocorticoid receptor. In these experiments we sought to determine whether physiological replacement of endogenous B would restore immunohistochemical staining patterns of CRF and VP in the paraventricular nucleus (PVN). Four groups of male, SD rats (n=3-4/group) were either sham-adrenalectomized or adrenalectomized and implanted with 0, 25 or 75% B:cholesterol pellets. Because staining with both antibodies is sparse in intact rats and still faint in adrenalectomized rats, 72h after surgery, all rats were reanesthetized and injected intraventricularly with 75 µg colchicine. Blood samples for B determination were collected immediately before colchicine treatment and 48h later, just prior to perfusion with saline followed by Zamboni's fixative. Body and thymus weight were recorded. Twelve 1-in-10 10 µm cryostat sections were collected and adjacent sections were stained for either VP or CRF. One section from each series was stained with thionin for latter mapping and reconstruction. Both VP and CRF sections were incubated at 1:1000 in 1% goat serum for 48h at 4 degrees and developed with the Vector ABC kit with 3,3'diaminobenzidine. Immunostaining of CRF and VP in control and adrenalectomized rats confirmed previous reports. Plasma B levels were 16.1±4.4 and 0.4±0.3 µg/100 ml, respectively and thymus weight was also significantly different (330±21 v 474±27 mg/100g bw, p 0.05). Plasma B levels of 6 ug/100 ml over the 5d period abolished VP staining the the parvocellular cells and normalized the distribution and intensity of CRF staining to levels seen in the controls. High B treatment resulted in plasma levels of 18.9±5.8 µg/100 ml, induced thymic atrophy (100±34 v 330±21 mg/100g bw of sham) and markedly decreased CRF staining in parvocellular cells of the PVN. The differential sensitivity of VP and CRF immunostaining as a function of plasma B suggests that adrenalectomy-associated increase in VP staining in the PVN may be a result of the lack of assoication of B with the type I high affinity corticosteroid receptor, whereas CRF staining may be modulated by association of B with the type II, glucocorticoid receptor. (Supported, in part, by AM28172, HL29714, and a UCSF Academic Senate Research Grant. SFA also expresses appreciation to the Kroc Foundation.)

450.7 HYPOTHALAMIC CORTICOTROPIN-RELEASING FACTOR IMMUNOREACTIVITY IS REDUCED BY EITHER GONADECTOMY OR CHRONIC ESTROGEN TREATMENT. D.A. Haas, B. Borgundvaag and S.R. George, Departments of Medicine and Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8, Canada.

Corticotropin-releasing factor (CRF) has been implicated to have an influence on the reproductive system. However, since the role of gonadal steroids in the regulation of CRF is not known, this study was conducted to examine their effect. Hypothalamic CRF-like immunoreactivity (CRF-ir) was determined using a radioimmunoassay specific for rat CRF. Adult male rats underwent either orchidectomy or a sham procedure. Two weeks later the orchidectomized rats were administered testosterone propionate (20 mg/kg/day,s.c.) or vehicle for 1 or 3 days while the sham-treated group was given vehicle (n=7 per group). Orchidectomy resulted in a significant ($p<0.025$) decrease in CRF-ir compared to sham-treated rats (100.0±16.6 pg/mg vs 138.9±4.6 pg/mg). Testosterone administration did not alter CRF-ir levels significantly from vehicle treatment in orchidectomized rats. Adult female rats sacrificed 3 weeks after ovariectomy (n=15) were found to have significantly ($p<0.025$) decreased CRF-ir compared to intact females (n=15) (84.5±8.3 pg/mg vs 110.7±8.5 pg/mg). Administration of β-estradiol-3-benzoate (E) (50 µg/day, s.c.) for periods up to 5 days did not significantly alter CRF-ir in ovariectomized females. In order to study the effects of chronic E administration, female rats were ovariectomized and surgically implanted with silastic pumps containing 2.5 mg E (n=41) or nothing (n=40), and then sacrificed at 3, 8 or 12 weeks after surgery. CRF-ir was significantly reduced in the E-treated groups at 3 (100.3±9.2 pg/mg vs 67.2±5.3 pg/mg, $p<0.005$) and 12 weeks (90.4±12.5 pg/mg vs 46.3±4.3 pg/mg, $p<0.01$), with no significant difference noted at 8 weeks. Intact females which were treated with E for 12 weeks also had reduced CRF-ir (51.8±13.0 pg/mg). These data show that hypothalamic CRF-ir was reduced by gonadectomy in both male and female rats. Acute treatment with gonadal steroids could not reverse this decrease in either sex. Chronic treatment with E decreased CRF-ir even further in ovariectomized rats, but also decreased CRF-ir in intact females. The combination of these results imply that the gonadectomy-related decrease in CRF-ir was not directly mediated by the loss of gonadal steroids, but by some other factor, possibly gonadal in origin.

450.8 CORTICOTROPIN RELEASING HORMONE AND OXYTOCIN STIMULATE THE SECRETION OF IMMUNOREACTIVE β-ENDORPHIN FROM HUMAN PLACENTA IN VITRO. A. N. Margioris,* M. Grino,* P. Protos,* P.W. Gold and G.P. Chrousos. (Spon: J.R. Glowa)DEB, NICHD, NIH and BPB, NINH, Bethesda, MD 20892, and Holy Cross Hospital, Silver Spring, MD 20901

Plasma immunoreactive (IR) corticotropin releasing hormone (CRH) increases progressively during pregnancy and rises further during labor. At parturition plasma immunoreactive oxytocin (OT) also rises significantly. Immunoreactive β-endorphin and IR-CRH are present in human placenta homogenates. A propiomelanocortin-like mRNA has been described in extracts of human placenta and we have recently isolated a single RNA species hybridizing with a human CRH probe and exhibiting the same size as rat hypothalamic CRH mRNA.

To investigate the relationship of CRH, OT, and placental IR-β-endorphin we have developed an in vitro perfusion system utilizing fresh human term placenta fragments. After a 60 min equilibration period the rate of peptide secretion became constant for at least 4 hours. The secretion rate of IR-CRH was 135.5±5.5 (mean ±SE, n=68) pg/5min fraction/g tissue and of IR-β-endorphin 7.7±1.3 (n=30) pg/5min fraction/g tissue. The molar ratio of secreted IR-CRH to IR-β-endorphin was approxiamtely 10.1. On Sephadex G-75 chromatography, the secreted IR-CRH exhibited the same apparent molecular weight as synthetic human CRH and the secreted IR-β-endorphin as synthetic human β-endorphin.

A 10 min exposure of the perfused placenta fragments to 10^{-8} and 10^{-6} M of synthetic human CRH or synthetic OT produced an immediate, sharp, and dose-dependent increase of the secreted IR-β-endorphin which lasted for about 20 min. OT had no effect on IR-CRH secretion at neither concentration employed. Exposure of the placenta fragments to depolarizing concentrations of KCL (56 mM) at the end of each perfusion produced an immediate 10 fold increase of IR-CRH.

We conclude that IR-CRH and IR-β-endorphin are secreted from human placenta fragments in vitro; both CRH and oxytocin stimulate the release of IR-β-endorphin from human placenta; the oxytocin-stimulated IR-β-endorphin release is not CRH-mediated. These findings suggest that the human placenta may participate in the physiological adaptation to the stress of labor and delivery.

450.9 PLATELET ACTIVATING FACTOR STIMULATES HYPOTHALAMIC CORTICOTROPIN RELEASING HORMONE SECRETION IN VITRO

R. Bernardini*, A.E. Calogero*, G.P. Chrousos, C. Saoutis* and P.W. Gold* (SPON: G.R. Merriam). DEB/NICHD and BPB/NIMH, Bethesda, MD 20892.

Products of the immune system may interact with the hypothalamic-pituitary-adrenal (HPA) axis. During allergic or inflammatory reactions, platelet activating factor (PAF), a glycerolipid with potent platelet aggregating properties, is released by neutrophils, eosinophils and basophils. To examine the hypothesis that PAF constitutes a link between the immune system and the HPA axis, we investigated the ability of this chemical to stimulate immunoreactive corticotropin releasing hormone (iCRH) secretion by explanted rat hypothalami cultured in vitro. Single explanted rat hypothalami were incubated overnight in medium 199 containing 0.1% BSA and 20 uM bacitracin. The following day the hypothalami were exposed to PAF at concentrations ranging from 10^{-10}M to 10^{-5}M for 40 min. Each hypothalamus was finally exposed to 60 mM KCl to test the viability of the tissue. A minimum of 6 hypothalami per group were examined. ANOVA and Duncan multiple range test were performed on logarithmically transformed data. PAF stimulated iCRH secretion at concentrations ranging between 10^{-9}M and 10^{-6}M. The most effective concentration was 10^{-8}M ($p<0.01$ vs basal secretion), inducing a 300% increase of iCRH above baseline values. To examine whether prostanoids are involved in PAF-induced iCRH response, we evaluated the ability of indomethacin, a cyclooxygenase inhibitor, eicosatetraynoic acid (ETYA), a cyclo- and lipoxygenase inhibitor and nordihydroguaiaretic acid (NDGA), a lipoxygenase inhibitor, to inhibit this response. Each of the inhibitors was added 2 h prior to and during treatment with 10^{-8}M PAF. In addition, alprazolam and diazepam, two known inhibitors of iCRH secretion, were added separately to the media 20 min before and during exposure to 10^{-8}M PAF. The PAF-induced iCRH secretion was completely prevented by treatment with indomethacin and ETYA ($p<0.01$ vs 10^{-8}M PAF), but not by NDGA. Alprazolam (10^{-6}M), but not diazepam, also inhibited PAF-induced iCRH secretion ($p<0.01$). In summary, PAF increases iCRH secretion by single explanted rat hypothalami. This action may represent another link between the immune and the endocrine system. The PAF-induced iCRH secretion is probably mediated by prostaglandins, but not leukotrienes, and can be blocked by alprazolam, a triazolobenzodiazepine. From these results we speculate that PAF may stimulate secretion of glucocorticoids in vivo and, hence, cause an inhibition of its own secretion via glucocorticoid-induced suppression of phospholipase A_2. Immune system activation and secretion of PAF might cause variable degrees of HPA axis activation, depending on the secretory status of the hypothalamic CRH secreting neurons.

450.10 STIMULATORS OF ADENYLATE CYCLASE AND PROTEIN KINASE C IN MIDBRAIN CENTRAL GRAY FACILITATE LORDOSIS. C. V. Mobbs and D. W. Pfaff. (SPON: Z. Wenzel). Rockefeller University, New York, NY 10021.

Female rats exhibit the reflexive posture lordosis in response to flank pressure. Estrogen must be present for the stimulus and the response to be coupled; therefore this system may be useful to examine mechanisms of neuromodulation. Estrogen acts on the ventromedial nucleus of the hypothalamus, apparently inducing the synthesis of polypeptides which are transported to the midbrain central gray (MCG). LHRH and substance P, when infused into the MCG, have been shown to enhance lordosis in rats given low levels of estrogen to induce minimal lordosis. Since these peptides and other peptides have been shown in some cases to act through cAMP and/or protein kinase C, and since these second messenger systems have been implicated in neuromodulation, we examined if stimulating these second messenger systems in the MCG would enhance lordosis. Ovariectomized female Fisher rats were given bilateral cranial cannulae targeted to the MCG. One week later they were injected s.c. with 1-2.5 ug estradiol benzoate (dose adjusted for each rat to result in moderate lordosis). Forty-eight hours later 1 ul of forskolin (5 ug/ul 50% DMSO) or phorbol-20-oxo-20-deoxy-12,13-dibutyrate (PBu; 5 ug/ul 50% DMSO), which stimulate adenylate cyclase and protein kinase C respectively, were infused into the MCG. Some animals were also given 1 ul containing both agents (2.5 ug forskolin+2.5 ug PBu/ul 50% DMSO) or just 50% DMSO. Lordosis in response to manual stimulation was assessed just before infusion, just after infusion, then 15, 30, 45, 60, 90, 120, 180, and 240 minutes after infusion. After completion of experiments, brains were examined for cannula placement. Both forskolin and PBu facilitated lordosis, with maximum stimulation occuring either at 60 or 90 minutes; by 2 hours lordosis was back to pre-infusion levels. Lordosis scores just before (Pre) and when lordosis was maximum (Post) are shown below; statistical comparisons were based on the Mann-Whitney U test.

	Forskolin	PBu	Forsk+PBu	50% DMSO
n=	9	9	5	5
Pre	1.4 ± 0.03	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
Post	2.0 ± 0.2(a)	2.1 ± 0.2(a)	2.7 ± 0.1(a,b)	1.3 ± 0.06

(a) $p<0.01$ post vs. pre; (b) $p<0.05$ vs. post-forskolin

We conclude that in the MCG both cAMP and protein kinase C may be involved in the control of lordosis. Since the two systems may synergize, they could mediate different peptide effects.

450.11 INCREASED PLASMA OSMOLALITY PRODUCES INCREASED LEVELS OF GTP-BINDING PROTEINS' Gs AND Gi α-SUBUNIT mRNAs IN THE PARAVENTRICULAR AND SUPRAOPTIC NUCLEI. W. S. Young, III, R. Burch* and E. Shepard*. Lab. of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892.

The paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus contain magnocellular neurons, some of which synthesize vasopressin, transport it to the posterior pituitary, and subsequently release it to regulate salt and water balance. Increases in plasma osmolality brought about by drinking 2% salt water lead to increased vasopressin biosynthetic activity evidenced by elevated vasopressin mRNA levels in the PVN and SON and enhanced vasopressin release in rats. There is evidence for both local and distant osmoreception but the mechanisms which regulate subsequent vasopressin synthesis are not well understood. As a first approach to studying intracellular control of vasopressin gene expression, we examined several aspects of second messenger systems after salt-loading.

Increased plasma osmolality could influence the cAMP system. Using in situ hybridization histochemistry, we found relatively high levels of RNA transcripts encoding the GTP-binding protein α-subunits (Gs, Gi, and Go) and the catalytic and regulatory subunits of cAMP-dependent protein kinase (Cα and RIα) in the PVN and SON of Sprague-Dawley rats. Binding of ^{3}H-forskolin, a marker of the Gs-adenylate cyclase complex, was also detected in the PVN and SON by in vitro "receptor" autoradiography. One week of drinking only 2% saline led to 66% (n=12, p=0.021, two-tailed t-test) and 122% (n=12, p=0.002) increases in Gsα mRNA in the PVN and SON, respectively. Similarly, 70% (n=12, p=0.046) and 123% (n=10, p=0.029) increases in Giα mRNA were seen. No significant changes in Go, Cα, or RIα transcripts nor in forskolin binding were observed.

These data suggest that if the changes in Gsα and Giα mRNAs lead to increased levels of their encoded proteins, then elevated cAMP levels could result from increased coupling of Gsα to adenylate cyclase facilitated by removal of the inhibitory βγ-complex by Giα. Indeed, initial results after salt-loading show that basal cAMP levels in fresh tissue punches of SON (PVN was not examined) increased 60% (1.5±0.3 to 2.4±0.8 pmol/punch±S.D.; n=20, $p<0.0001$) while cholera toxin-stimulated cAMP levels increased 30% (24±3 to 31±5 pmol/15min/punch±S.D; n=20, p=0.006) supporting the idea that Gsα is rate-limiting in this context. We are further examining the significance of the elevated G-protein mRNAs by measuring levels of G-proteins themselves.

450.12 ENKEPHALIN mRNA IN THE HYPOTHALAMIC PARAVENTRICULAR AND SUPRAOPTIC NUCLEI OF THE RAT. S.L. Lightman and W.S. Young III. Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, Maryland 20892, USA.

There is much data implicating endogenous opioid peptides in the control of pituitary function. The source and physiological control of these peptides is, however, unclear. We previously described changes in supraoptic (SO) and paraventricular (PV) dynorphin mRNA in response to an osmotic stimulus (Lightman, S.L. and Young, W.S. J. Physiol. In Press) and have now investigated the control of enkephalin mRNA in these nuclei.

Four groups of experimental animals were studied: (i) Drinking water was replaced with 2% saline for up to 12 days; (ii) 1.5M hypertonic saline was injected i.p.; (iii) lactating mothers were studied 10 days post partum; and (iv) opiate withdrawal was precipitated by naloxone after chronic administration of morphine for 12 days (total dose 670mg/kg). Experimental animals and their controls were decapitated, the brains rapidly removed and placed on dry ice. 12μ sections were cut through the PV and SO nuclei and thaw-mounted on gelatine coated slides. Hybridisation was performed with a synthetic 48 oligonucleotide probe, complementary to the enkephalin precursor sequence, which was labelled using ^{35}S-dATP and terminal deoxynucleotidyl transferase. Sections were then either apposed to Kodak X-Omat AR film or dipped in NTB3 emulsion for higher resolution.

Although there was marked hybridisation of the enkephalin probe to perifornical cells and in the striatum, control animals have only very few parvocellular cells containing detectable enkephalin transcripts, and no detectable message in the magnocellular areas. In spite of the possibility that enkephalin might coexist in oxytocin magnocellular cells, lactation was not associated with the appearance of detectable magnocellular enkephalin mRNA. After 12 days drinking 2% saline however, occasional SO and PV magnocellular cells did contain detectable message. The animals undergoing the stress stimuli of i.p. hypertonic saline and opiate withdrawal showed very marked changes. Not only did both these groups of animals have many magnocellular neurons which were lightly labelled on microscopic examination, but they both responded with a massive increment of enkephalin mRNA over the parvocellular PV nucleus, to a degree that could be clearly quantitated on film. This occurred within two hours of opiate withdrawal and at four hours after hypertonic saline. The cells containing enkephalin mRNA are in the same area as the CRF containing parvocellular cells. These data suggest that transcription of enkephalin mRNA in the hypothalamus may play an important role in the neuroendocrine response to stress.

451.1 TEMPORAL INTERACTIONS INVOLVED IN DIRECTION-SELECTIVITY OF STRIATE CORTICAL NEURONS OF THE CAT. Curtis L. Baker, Jr. and Max S. Cynader, Department of Psychology, McGill University, 1205 Ave. Docteur Penfield, Montreal, Quebec, Canada, H3A 1B1; and Dept. of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1

Flashed bar-shaped stimuli were used to study direction-selective neurons in striate cortex of the cat. Stimuli flashed sequentially at two adjacent points in the receptive field produced direction-selectivity comparable to that elicited by continuously moving stimuli.

Previous studies have shown that each neuron has a characteristic optimum spatial displacement (Dopt) between the 2 flashes to produce maximal direction-selectivity. This value of Dopt was shown to be independent of the temporal interval used in its measurement, and linearly proportional to the spatial subunit wavelength (reciprocal of best spatial frequency) for a given neuron. Attempts to measure a comparable optimum temporal separation between 2 flashes were frustrated by the finding that most striate cortical neurons respond only to prolonged flash exposure durations. A given neuron's "integration time" was found to be inversely proportional to Dopt and to the spatial subunit wavelength. The optimum velocity for direction-selectivity in a given neuron appears to be jointly determined by the spatial properties (Dopt or spatial frequency) and the integration time.

To probe the temporal conditions needed for 2-flash direction-selectivity, a neuron's directional response was measured for a series of values of temporal stimulus onset asynchrony (SOA) between 2 flashes delivered at a constant spatial displacement (the Dopt measured for that neuron). These measurements were made for a series of values of flash exposure durations, with all experimental conditions randomly interleaved.

Some neurons showed direction-selectivity for a broad range of SOA's with little dependence on flash exposure duration. Other neurons exhibited some selectivity for a constant interval between the end of the first flash and the beginning of the second flash. Correlation of this qualitative heterogeneity with other cell properties is not yet evident.

These data may be interpreted in terms of different kinds of input signals (e.g., transient vs sustained) to Reichardt-type correlation models, and suggest that direction-selectivity in striate cortex neurons may arise independently from nonlinear directional interactions between several different combinations of inputs.

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451.2 MOVEMENT MODELS AND DIRECTIONALLY SELECTIVE NEURONS IN THE CAT'S VISUAL CORTEX. R.C. Emerson, J.R. Bergen*, and E.H. Adelson*. Center for Visual Science, U. Rochester, Rochester, NY 14627; SRI-David Sarnoff Research Center, Princeton, NJ 08540; and MIT Media Lab, Cambridge, MA 02139.

In striate cortical receptive fields of the cat, we have analyzed nonlinear response interactions between pairs of optimally oriented bars that represent samples of movement. Pairs were drawn from a randomized spatiotemporal bar sequence that included a wide range of separations in space (Δs) and time (Δt) to test a wide range of velocities. We have found that directionally selective striate neurons display nonlinear facilitation for responses to movement sequences in the preferred direction and nonlinear suppression for sequences in the null direction.

To gain insight into the connectivity of neurons performing this feat, we simulated a two-bar superposition test for two different models that have been proposed to explain movement processing, the Reichardt model and the opponent energy model. Our measured nonlinear interaction disagreed with the nonlinear residual of the simulated superposition test at the final "opponent" output of both models. The measured cortical interaction for one movement direction did not always invert for the sequence with Δs and Δt values of the same magnitude (same equivalent speed), but in the opposite direction, as required by the models. This same disagreement existed between measured cortical interactions and simulated interactions at the half-detector stage of the Reichardt model, the stage immediately preceding the opponent output, because spatiotemporally tuned Reichardt detectors produce an inherent opponency even before the final output stage.

However, the lack of opponency in the measured interactions agreed closely with the simulated interaction for the preopponent stage of the energy model, the stage at which separate "energy" for opposite directions is represented. Furthermore, the energy-model simulation agrees with our finding that in directionally selective striate neurons the Δt for maximal interaction is proportional to the Δs between the bars, i.e., the interaction is highly inseparable in space and time, and shows a strong velocity preference.

Thus, our directionally selective cortical neurons match closely the properties at the preopponent stage of the energy model. This observation suggests (1) that these striate neurons do not carry fully motion-opponent information, and (2) that these cells may depend on strongly inseparable peripheral linear filters that are similar to spatiotemporal Gabor filters. (Supported by EY04630, EY06679, and EY01319.)

451.3 ADAPTATION OF SINGLE UNITS IN CAT VISUAL CORTEX: AFTEREFFECTS AS A FUNCTION OF DIRECTION, WITH IMPLICATIONS FOR CORTICAL ORGANIZATION. Saul, A.B. and Cynader, M.S., Dept. of Psychology, Dalhousie University, Halifax, N.S., Canada, B3H 4J1

We employed an adaptation paradigm in which each 5 second test stimulus presentation was immediately preceded by 15 seconds of adapting stimulation. All stimuli were drifting gratings of varying contrast, direction, and spatial and temporal frequency. These techniques are sensitive to aftereffects: in about 100 runs on 25 cortical cells, we observed selective adaptation in every run.

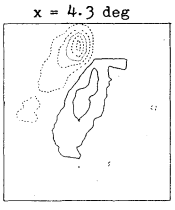
The adaptation results have implications for cortical connectivity. The mechanism underlying direction selectivity is believed to involve inhibitory connections. One might suppose that this inhibition arises from mutual antagonism between direction selective cells preferring opposite directions of motion. Adapting with a high contrast grating drifting rightward drastically reduces the responses of cells which prefer rightward motion. If these cells normally inhibit a cell preferring leftward motion, rightward adaptation would reduce this inhibitory input. The disinhibition would show up as an enhanced test response in a cell's null direction following adapting in the null direction. We have found, to the contrary, that adaptation aftereffects are bidirectional, and have never observed enhanced responses in the null direction following adapting in the null direction.

These bidirectional aftereffects are also temporal frequency selective. The tuning of adaptation in the temporal domain is often surprisingly sharp, with octave bandwidths. Both directions of motion have similar tuning of adaptation, although sometimes the magnitude of the aftereffect is reduced in the direction opposite that adapted. We have found that the tuning of aftereffects depends on the adapting stimulus, implying that the mechanisms underlying adaptation involve the inputs to a cell, rather than intrinsic properties of the cell alone. Since aftereffects are bidirectional and temporal frequency selective, we suggest that direction selective cells receive bidirectional, temporally tuned inputs. Direction selectivity can be generated from such inputs through spatiotemporal phase specific interactions.

In experiments where we varied the adapting contrast, the contrast threshold for inducing aftereffects matched the cell's contrast threshold. The results of these experiments can be used to argue for inhibitory mechanisms underlying adaptation. Modification of inhibitory gain offers an explanation for why adaptation reduces cortical responsiveness without inducing a compensatory disinhibition. Increasing the strength of mutually inhibitory connections between cells with similar response properties would tend to reduce responsiveness uniformly, as we have observed, rather than enhancing previously null responses.

451.4 **SPATIOTEMPORALLY ORIENTED SIMPLE RECEPTIVE FIELDS: LOCAL LINEAR MOTION DETECTORS** J. McLean*, S. Raab* and L. Palmer. Univ. of Pennsylvania, Phila., Pa. 19104

We and others have demonstrated that simple cells linearly summate stimuli distributed in 2D space. However, responses to moving patterns are often highly asymmetric depending on the direction of motion. Traditionally, this has been viewed as a spatiotemporal nonlinearity dependent on mechanisms such as inhibition propogating ahead of the stimulus in the null direction. More recent models of motion perception utilize filters which are linear but oriented in space-time. We have developed a variation on such a linear model (after Adelson & Bergen) which accounts for responses of simple cells to moving stimuli. To test this model, we have estimated the 3D (space-space-time) impulse response of simple cells by crosscorrelating the spike train with a white noise stimulus ensemble. This measurement was used to predict the optimal direction and velocity of moving stimuli using the convolution integral. These predictions agree closely with experimentally aquired responses to moving stimuli for the majority of simple cells. A contour plot of a section through the 3D impulse response of a simple cell is shown here. The x-axis is the width axis of the field which consists of two subregions, one driven by bright stimuli (solid lines) and the other by dark (dashed lines). These subregions, which are elongated, parallel and oriented in space-time determine the optimal velocity (7°/sec) and direction (L to R) for moving bars. Since, orientation of bright and dark subregions were similar, direction preference was the same for both bright and dark moving bars. We conclude that most simple cells are roughly linear in space-space-time and that it is orientation of the subregions in space-time which confer directionally asymmetric responses to moving stimuli.



Supported by BNS-8420402.

451.5 DIRECTIONAL SELECTIVITY OF SIMPLE CELLS IN CAT STRIATE CORTEX RESULTS FROM LINEAR SPATIAL SUMMATION. I. (SPON: J. Gordon) Robert E. Soodak*, R. Clay Reid* and Robert M. Shapley. The Rockefeller University, N.Y., NY 10021.

In a previous report (Soodak, PNAS 83:9259, 1986) a receptive field model of cortical simple cells was presented which accounted for both the sharp orientation tuning and directional selectivity of these neurons. In this model, simple cell receptive fields are specified as a linear summation of Gaussian subunits, which can be thought of as representing converging afferents from the LGN. The ability of this model to account for the directional selectivity of simple cells suggested that linear spatial summation might be the basis of this phenomenon. We have investigated the aspects of simple cell receptive fields that relate to their directional selective properties, and compared these results to predictions of the model.

The experimental paradigm consisted of measuring the directional selectivity with drifting sinusoidal gratings and comparing this with the responses to stationary contrast-modulated stimuli. The stationary stimuli used were narrow bars and gratings, and their contrast was modulated sinusoidaly with time. Both response amplitude and response phase were measured as a function of position of the stimulus in the receptive field. Consideration of the dependence of response phase on the position of the stimulus was especially important. In the proposed model, directional selectivity is due to construction of receptive fields from subregions that are not constrained to respond at either 0 deg temporal phase delay (pure on response) or 180 deg temporal phase delay (pure off response). A linear receptive field with this constraint will have no directional selectivity, and its response phase will be invariant with position of a stationary contrast-modulated stimulus. In contrast, a receptive field with directional selectivity due to linear spatial summation will show a continuous variation of response phase with position of both stationary bar and grating stimuli. For most simple cells examined, there was a qualitative agreement between the responses observed, and those expected from a neuron with directional selectivity arising from linear spatial summation. In cells with little or no directional selectivity, the response phase to bar and grating stimuli was relatively invariant with position. Those cells with strong directional selectivity showed continuous variation in response phase with position, and the direction of change was always in the predicted direction.

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451.6 DIRECTIONAL SELECTIVITY OF SIMPLE CELLS IN CAT STRIATE CORTEX RESULTS FROM LINEAR SPATIAL SUMMATION. II. R. Clay Reid*, Robert E. Soodak* and Robert M. Shapley. The Rockefeller University, New York, NY 10021.

When stimulated with contrast reversing gratings, any linearly summating directional receptive field (RF) should show continuous variation in response phase as the position of the grating is varied. More quantitatively, it can be shown that the amplitude and phase of the responses should follow a simple functional form, most simply modeled as an ellipse in a polar plot of amplitude and phase (Movshon et al., 1978). Our data from simple cells show an excellent fit to this form, arguing for a high degree of linear spatial summation for non-directional stimuli. Further, from the parameters obtained by fitting this function, a quantitative prediction of the directional behavior is obtained. To test this, gratings of the same spatial frequency, temporal frequency, and contrast as the contrast reversing gratings were drifted in the preferred and non-preferred direction. We have found a very high correlation between the predicted directionality from the contrast-reversing gratings and the response to drifting gratings, often predicting >50% of the actual directionality. Studies parametric in spatial and temporal frequency show that the directionality varied with these parameters, often decreasing at higher temporal frequencies, a variation which was mirrored in the prediction from the static gratings.

The linear mechanisms of directional selectivity were robust even in small regions of the RF. First, when the RF was masked except for a narrow window entirely within an "on" or "off" region, the cells still showed directional selectivity to sinusoidal gratings when even less than a quarter of a cycle was visible at any time. Second, when static narrow bars (rather than sine-wave gratings) were modulated sinusoidally at various locations in the RF, the temporal phase of response varied continuously across the RF. Studies parametric in temporal frequency show that this phase change does not seem to be due to a simple delays, but to more complex changes in temporal integration with position. Therefore, rather than describing the simple cell RF in terms of on and off regions (even with variable temporal delays), a more complex characterization of the temporal characteristics is necessary. In conclusion, we have found that the temporal response characteristics of some simple cells vary continuously along the RF and that this variation explains a good deal of the directionality of these cells.

Supported by NIH 2 T32 GM 07739 and EY 1472.

451.7 SENSITIVITY OF CAT CORTICAL NEURONS (AREAS 17, 18, 19, PMLS) TO INTERACTION BETWEEN OBJECT AND BACKGROUND - LOCAL vs. GLOBAL PROCESSING REVISITED. H.R.O. Dinse and K. Krüger (SPON: A. Schoppmann) Dept. of Zoology III, Biophysical Section, Univ. of Mainz, D-6500 Mainz, FRG

While quite a lot is known about how neurons analyze objects, which reflects the local aspects of visual information processing, far less is known concerning the analysis of interactions between object and background and the contribution of the different visual areas to this task.

Neurons in the visual areas 17, 18, 19 and PMLS were stimulated with a bar defined as an object superimposed with a large field visual noise (2-dimensional Gaussian white noise covering 50 x 50 degrees of the visual field) defined as background. Both were moved with different relative velocities, inphase or antiphase to each other.

Using this method, the activity of all neurons tested in each area was affected. It was possible to fit these neuronal effects into a scheme based on the assumption that the following parameters resulting from object-background-interaction have to be determined by neuronal systems: (1) motion, (2) velocity and (3) direction of the background, and (4) relative motion, (5) relative velocity and (6) phase between object and background. Indeed, six types of neurons, each sensitive to one of these parameters, were found in all areas tested, though in different percentages.

Systematically masking out different parts outside of the classical receptive field provided evidence that these parts exert a strong excitatory or inhibitory modulatory effect on the cell's response to the object. Although it is not possible to elicit neuronal activity from these regions alone, they reveal stimulus specific properties, which have up to now been attributed to the excitatory regions of receptive fields only.

Three important implications are briefly mentioned: (1) Direction selectivity of a cell depends in 70 % of all neurons on the background status. (2) The recently proposed double opponent mechanism for PMLS neurons might be a special case of a more general receptive field organization already present in the primary visual areas. (3) Finally, global processing seems not to be restricted to hierarchically higher substrates, but common even at the level of the primary visual areas.

(Supported by Deutsche Forschungsgemeinschaft)

451.8 Lesions of the Lateral Suprasylvian cortex affect speed but not direction discrimination. Tatiana Pasternak and John Maunsell, University of Rochester Medical School and Center for Visual Science, Rochester, NY 14627.

The majority of neurons in the cat lateral suprasylvian (LS) cortex are directionally selective and respond well to moving stimuli over a wide range of temporal frequencies. These properties suggest that LS is well suited for analyzing image motion. To examine the role of LS in motion perception we tested the ability of cats to discriminate differences in stimulus direction and speed before and after making bilateral lesions in that region of cortex using ibotenic acid.

Preliminary results have been collected from one animal with histologically confirmed lesions of LS. Several small lesions were made along the posterior lateral bank of the sulcus on both sides, extending from 2P to 10A. Within this range about 30% of the medial bank was lesioned, with minor involvement of the lateral bank. Contrast sensitivity for detecting moving gratings was unaffected by the lesions. The cat was also able to discriminate grating direction at threshold contrasts over a wide range of temporal frequencies (2-10 Hz). However, its ability to discriminate small differences in speed was normal only at low temporal frequencies (2.2 Hz). At higher frequencies, speeds could be discriminated only at high grating contrasts (>24%). Above 4.5 Hz, speed discrimination could not be measured even at very high contrasts. By manipulating spatial frequency of the gratings we determined that these deficits in speed discrimination were specific to high temporal frequencies, not to high speeds, i.e. the cat could discriminate speeds even at a relatively high base speed (16 deg/sec) as long as the gratings were of low temporal frequency (<4 Hz).

These preliminary results suggest that cortex in the lateral suprasylvian sulcus may be involved in the analysis of stimulus speed but not necessarily direction. Thus, direction and speed may be processed independently at later stages of motion analysis.

Supported by EY06175 and EY01319.

451.9 AREAS 17 AND 18 CONTRIBUTE THE MAJOR FUNCTIONAL INPUT TO TWO SUPRASYLVIAN CORTICAL AREAS IN THE CAT DURING THE GENERATION OF OPTOKINETIC NYSTAGMUS. S.J. Herdman, R.J. Tusa and C.B. Smith, Johns Hopkins Hospital, Baltimore MD 21205 and Lab of Cerebral Metabolism, NIMH, Bethesda MD 20892.

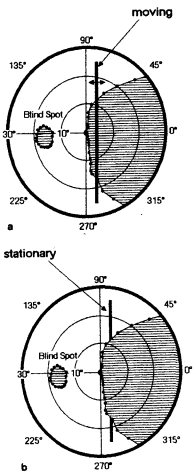
In order to better define the cortical regions involved in optokinetic nystagmus (OKN), two techniques have been employed: determination of local rates of glucose utilization during the generation of OKN (autoradiographic 2-deoxyglucose method of Sokoloff et al 1977) and ablation of restricted portions of cortex. Four groups of animals were used: normal cats viewing a stationary OKN drum (N=3), normal cats generating OKN (N=3), cats with a unilateral 17 and 18 plus corpus callosum lesion generating OKN (N=2) and in one cat with a unilateral 17 and 18 lesion generating OKN. In all cats generating OKN the drum was rotated in one direction at approximately 40 /sec.

There was an increase in metabolic activity in discrete portions of visually responsive cortex in normal cats generating OKN. These regions coincided with areas 17 and 18 and four areas in suprasylvian cortex (21a, 21b, PMLS and VLS). Unilateral ablation of the suprasylvian areas has previously been shown to decrease OKN gain (slow phase eye velocity/target velocity) to stimuli moving toward the side of the lesion (Neurosci. 9:154, 1983). Unilateral ablation of areas 17 and 18 did not decrease OKN gain nor did it affect the metabolic activity of the suprasylvian areas. Unilateral ablation of areas 17 and 18 plus section of the corpus callosum, however, resulted in a decrease in OKN gain toward the side of the lesion. This lesion also decreased the metabolic activity in areas 21a and PMLS ipsilateral to the 17 and 18 ablation but did not decrease the metabolic activity in areas 21b and VLS.

The results suggest that in normal cats generating OKN, the increase in metabolic activity in areas 21a and PMLS depends on an input from areas 17 and 18 either ipsilaterally or through the corpus callosum. This increase in metabolic activity may reflect sensory information used in the generation of OKN.

451.10 MOVEMENT-DEPENDENT PERCEPTUAL COMPLETION ACROSS A CORTICAL SCOTOMA. E.Pöppel, P.Stoerig and G.deLangen. Dept. of med.Psychology,Goethestr.31,8München 2, FRG

A lesion within the central representation of the visual field results in a homonymous field defect in the two eyes. Because of the particular locus of lesion in one such patient (see also: Pöppel, Naturwiss.72 (1985)599; Nature 320 (1986) 523) the question of perceptual completion across an area of cortical blindness could be studied. If a long stick is used as a stimulus, the patient



reports seeing separate parts of the stick in the right upper and right lower quadrant if the stick is kept stationary (see fig.). With jerky movements the patient reports seeing the entire stick. This stimulus completion appears to be easier with movements towards the fovea. Completion was also possible if the stick was moved behind an occluder; thus, no residual visual functions or "blindsight" are involved (Pöppel et al. Nature 243 (1973) 295). If the stick was seen by one eye in the upper and by the other eye in the lower quadrant, completion was still possible; thus the effect must be central. If the stimulus had one color throughout completion was easily obtained. This was not true when the color above and below the scotoma was different. Completion was also obtained for stimuli that had the form of concave or convex arcs. Geometrically more complex stimuli were, however, not completed There was also completion if the stimulus remained stationary and the eyes were moved between two targets. Taken together the results indicate that in this case completion was perceptual and not "cognitive". Hypothesis: A neuronal mechanism seems to be tapped by the moving stimulus within the functional region of the visual cortex that on the basis of local stimulus features (like color) projects trajectories throughout the visual field that under normal circumstances seems to be inhibited.

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(Sponsor: Poeppel)

451.11 MOTION-IN-DEPTH IN AN AMBIGUOUS FIGURE: EFFECT OF CYCLOPEAN SPATIAL FREQUENCY. J.V. Odom[1], G-M Chao[1]* and M. Fendick[2]*, [1]Department of Ophthalmology, WVU Medical Center, Morgantown, WV. 26506, [2]Foederer Eye Movement Center for Children, Wills Eye Hospital, and Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA. 19107

We have employed anaglyphic dynamic random element stereograms (ADRES) to generate square-wave horizontal gratings-in-depth. When the disparity of the gratings was reversed in a square wave manner, the gratings appeared to move in depth. However, movement appeared in either the frontal (x-y) plane or the depth (z) plane. The plane in which apparent movement ocurred depended on disparity. At smaller disparities gratings appeared to move in the z plane; at larger disparities grating apparent movement was in the x-y plane. Square-wave disparity reversal of square-wave gratings-in-depth is an ambiguous figure. Green and Odom (Science, 233:1426-1429, 1986) suggested that in ambiguous conditions objects appear to move to the most proximal location in x-y-z space. Therefore, we hypothesized that the disparity at which z-plane motion became x-y plane motion would depend on bar width of the gratings. We measured the threshold disparity for the change in the plane of motion employing seven stereo normal observers aged 26-43 years at four bar widths of 207, 138, 69 and 34.5 arc min. A double staircase procedure was used to determine thresholds, one staircase starting at a small disparity, the other at a large disparity. The log disparity at which motion direction changed was significantly correlated with log bar width across the seven subjects (r=0.632). The slope of the regression equation relating the disparity and bar size was significantly different from zero ($t(26)=4.159$, $p<.05$). The slope was approximately 0.5 indicating that on average a log unit change in threshold of change in motion from the z to x-y planes required a twofold change in log bar width. The results are qualitatively consistent with the hypothesis that the bars appeared to move to the nearest position in 3-D space, assuming that the dimensions are scaled approximately logarithmically. One may, also, interpret the slope of the function as a disparity gradient which limits perception of motion in the z plane. (cf. Burt, P. and Julesz, B., Science, 208:615-617, 1980). These results may be interpreted in terms of the characteristics of neurons responsive to cyclopean motion in depth (Regan, D. and Cynader, M. Invest. Ophthalmol. 4 Vis Sci, 22:535-550, 1982) We suggest that cells responsive to cyclopean apparent motion toward or away from the observer (z plane) operate over a more limited range of disparity gradients than cells responsive to cyclopean apparent motion in the frontal (x-y) plane. Supported in part by an unrestricted departmental grant from Research to Prevent Blindness.

451.12 CORTICAL PATHWAYS FOR MOTION ANALYSIS: CONNECTIONS OF VISUAL AREAS MST AND FST IN MACAQUES. D. Boussaoud*, L.G. Ungerleider, and R. Desimone. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Visual areas MST and FST in the dorsal bank and floor, respectively, of the caudal superior temporal sulcus (STS) both receive direct projections from MT, an area known to be related to motion analysis. The majority of cells in MST and a third in FST are directionally selective and many MST cells are also selective to the direction of tracking eye movements. To determine additional cortical components of this motion-analysis system, we injected multiple anterograde and retrograde tracers into physiologically identified locations within MST and FST of 5 cynomolgus monkeys.

The major connections of MST and FST are with each other and with areas in the STS and the posterior parietal cortex. In the STS, both MST and FST have connections with MT and with cortex of the dorsal bank of the sulcus including portions of the superior temporal polysensory area (STP), an area that contains many cells with complex directional selectivity. MST has particularly heavy connections with a region at the bottom of the dorsal bank, which may be either a part of STP or a new visual area. In contrast, FST is connected with an adjacent region in the floor of the sulcus, which may be a part of area IPa of Seltzer and Pandya ('78) or a new visual area. In the posterior parietal cortex, both MST and FST have connections with area V3A in the parieto-occipital sulcus, and with cortex in the intraparietal sulcus (especially MST) including but not limited to areas VIP and LIP. MST also has connections with medial parietal cortex including but not limited to area PO and the cingulate gyrus.

MST and FST have additional connections with area TF and the frontal eye field, and weak or inconsistent connections with the peripheral field representation of V4 and V4t, TEO, the posterior portion of the lateral sulcus, and area 7a on the inferior parietal gyrus. Connections were also found between the peripheral field representation of MST and that of V1 and V2, which may indicate some involvement of MTp at the injection sites.

The laminar pattern of connections suggests that MST and FST receive "forward" projections from areas MT, V3A, TF and, in the case of MST, area PO and the cingulate gyrus. In turn, MST and FST project "forward" to additional areas within the STS. The laminar pattern of connections with areas VIP and LIP in parietal cortex are closer to the "intermediate" type, as are the connections of MST and FST with each other.

The results of this and other studies suggest that the cortical pathway for motion analysis, which begins with the projection of V1 to MT, splits into at least two components. One component includes widespread regions of the posterior parietal cortex whereas the other extends into the temporal lobe, including several areas within the dorsal bank and floor of the STS.

451.13 ISOLUMINANT MOTION PROCESSING IN MACAQUE VISUAL AREA MT. T.D. Albright. Dept. of Psychology, Princeton Univ., Princeton, NJ 08544

Most models of neural motion detection involve establishing a correspondence between local image luminance levels that are displaced in space and time. Previous physiological studies have found substantial support for luminance-based mechanisms at various levels of the visual pathway. These models fail, however, to account for perceived motion of image elements that are not discriminable by luminance contrast; it is well known that motion can be perceived when viewing patterns that contain moving figures defined solely by isoluminant form cues such as motion, texture, stereoscopic depth or (under some conditions) chromatic contrast. We have studied motion sensitive cells of extrastriate visual area MT in an effort to determine whether this neural system mediates isoluminant motion processing. We now report physiological evidence of detectors for isoluminant motion, which exhibit form-cue invariant responses, i.e., responses that are invariant over different form cues.

We recorded activity from 49 MT neurons in macaques that were anesthetized with N_2O and immobilized. Our stimulus set included: 1) luminant bars that were either brighter or darker than background, 2) isoluminant motion bars defined by random or oscillatory motion of dots within a bar-shaped region, and 3) isoluminant texture bars defined by easily discriminable texture patterns that changed significantly across successive temporal frames. Stimuli were generated on a video display and moved through the classical receptive field in four directions across a static randomly textured background. Two-thirds of our sample of MT neurons that were selective for the direction of motion of conventional luminant stimuli also responded selectively to motion of one or more of the isoluminant stimuli. Moreover, for 90% of the cells that were tested systematically with multiple stimulus types and found selective for isoluminant motion, both response magnitude and strength of directional tuning were similar for all stimuli.

These findings indicate, first, that isoluminant motion perception may be mediated by a sub-population of MT neurons. Second, they imply that some form processing based upon isoluminant figural cues precedes motion detection in MT. Recent reports of V1 and V2 neurons sensitive to stimuli defined by stereo depth (Poggio et al., Vision Res., 25:397, 1985) or texture cues (DeYoe et al., Invest. Ophthalmol. Vis. Sci., 27:18, 1986) are consistent with this view. Third, form-cue invariance suggests a convergence of information from different form channels, either in MT or at some earlier stage. Lastly, these results identify MT with what has been referred to as the long-range motion system; as such, we should expect to find that MT directional selectivity is sensitive to a variety of stimulus configurational and contextual factors.

AGING AND DEMENTIA: FUNCTION II

452.1 LOCAL CEREBRAL GLUCOSE UTILIZATION DURING AGING IN THE RHESUS MONKEY. T.T. Soncrant*, H.W. Holloway*, D.M. Larson*, A.W. French* and S.I. Rapoport (SPON: G.P. Redmond). Laboratory of Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892, U.S.A.

Local cerebral glucose utilization (LCGU) was measured in 66 brain regions of 28 awake rhesus monkeys, aged 1 to 24 yr, by using the quantitative, autoradiographic [14C]deoxyglucose method. Under ketamine hypnosis, animals were restrained in a standard chair apparatus on the evening before the experiment. On the following morning, they were anesthetized with halothane 1% in oxygen, delivered by face mask. Femoral artery and vein catheters were placed surgically, and the skin incision was infiltrated with lidocaine and closed. Animals were chaired and allowed to regain consciousness in a light-and sound-controlled room. [14C]Deoxyglucose, 80 uCi/kg (50-55 Ci/mol), was administered intravenously 6 h later, and LCGU was measured during the subsequent 45 min incorporation period. Monkeys were grouped according to age: 1 yr (N=6), 3 yr (6), 9-12 yr (6), 15-18 yr (8), 24 yr (2). For each brain region, the mean LCGU value for each age group was compared to the 3 yr (young adult) mean, by using the Dunnett multiple comparison test.

No significant differences in mean LCGU were found between 1 and 9-12 yr in any brain region. Significant declines ($p < 0.05$) were found in only 9 of 66 regions in 15-18 yr monkeys (compared to 3 yr); three comparisons may have achieved statistical significance by chance. The areas in which LCGU declined significantly are frontal cortex, area 10, layers IV and V; frontal cortex, area 8, layer IV; motor cortex, layers III and IV; caudate nucleus; red nucleus; superior olive; inferior olive. LCGU was similar in 15-18 yr and 24 yr groups.

452.2 VISUAL ACUITY, MACULAR DEGENERATION AND PHOTORECEPTOR LOSS FROM THE RETINA OF THE AGED RHESUS MONKEY. T.M. Wengenack*, K.R. Brizee, J.M. Ordy, and W.P. Dunlap*. (Spon: R.J. Joynt) Univ. of Roch., Roch., N.Y. 14642, Tulane Univ., New Orleans, LA 70433, Pennwalt Corp., Roch., N.Y. 14623.

Visual acuity plays a fundamental role in eye-hand coordination, discrimination of fine spatial detail, and in all visually guided behavior of man and other diurnal primates. Remarkable progress has been made in vision research in clarification of visual acuity in relation to optical image forming mechanisms, the receptive field organization of the retina, and the geniculostriate system. Although acuity and retinal development have been studied more extensively in human and non-human primate infants and adults, only a very small number of studies have dealt with acuity, macular degeneration, and loss of photoreceptors from the retina of aging human or other diurnal primates. There is a paucity of information on acuity, macular degeneration and photoreceptor loss, particularly from the fovea and macula of the retina in man and in other higher diurnal primates. The specific aims of these ongoing studies were to evaluate age differences in visual acuity, funduscopic appearance of the macula, morphometric dimensions of the fovea, as well as loss of photoreceptor nuclei from the macula in young adult (4-7 years), middle aged (12-18 years), and aged (22-28 years) rhesus monkeys.

In cross-sectional age samples, asymptotic adult acuity norms for man and rhesus monkey have been estimated to be at least 20/20 in the Snellen notation, 1 minute of arc, or 35 c/dg of visual angle. In this study, a minimum separable binocular acuity of 0.83 min. of arc was observed in the young and an acuity of 0.86 min. of arc was observed in the middle aged monkeys. The acuity of 2.0 min. of arc was significantly poorer in the aged monkeys, ($F(2,8)=8.61$; $p = 0.01$). Fundus photography indicated macular changes in the aged monkeys comprising development of a granular appearance resembling drusen observed in aged human subjects. Morphometric evaluations of age differences in the width of the foveola and fovea indicated no significant age changes in widths along the horizontal meridian. However, the number of foveal cone inner segments of 39.0 per 100 μm in the aged monkeys was significantly lower than the mean of 44.2 in the middle aged monkeys ($t = 2.56$; $df = 5$; $p < 0.05$). The mean number of foveal cone nuclei also decreased significantly from 133.4 per 150 μm in the young to a mean of 112.0 in the aged monkeys ($F(2,8) = 4.3$; $p = 0.04$). There was also a significant age decrease in ganglion cell nuclear density along the horizontal meridian in the nasal quadrant from 60.8 per 150 μm in the young to 47.3 in the aged monkeys ($F(2,8)=4.2$; $p = 0.04$). The findings of this study indicated that the rhesus monkey may serve as a diurnal primate model for studies of aging in the visual system.

452.3 PERFORMANCE OF AGED RHESUS MONKEYS ON A VISUOSPATIAL TASK. M. Brickson*, J. Bachevalier, L. S. Watermeier*, L. C. Walker, R. G. Struble, D. L. Price, M. Mishkin, L. C. Cork*. The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; Lab. of Neuropsychology, NIMH, Bethesda, MD 20205.

Compared to young adults, a group of aged rhesus monkeys (Macaca mulatta) showed impairments on delayed nonmatching-to-sample and spatial delayed response (Presty et al., Soc. Neurosci. Abstr., 10:774, 1984; Presty et al., Soc. Neurosci. Abstr., 12:1312, 1986), abilities known to depend on visuo-limbic and prefronto-striatal systems, respectively. To analyze further the behavioral impairments associated with aging, we tested the same groups of monkeys on a visuospatial (route-following) task known to be sensitive to damage of yet another cerebral system, one involving the inferior parietal lobule and the banks and depths of the superior temporal sulcus (Petrides and Iversen, Brain Res., 161:63, 1979). Age ranges of animals at the start of this task were: Group I, 4-8 years; Group II, 17-20 years; Group III, 22-26 years; and Group IV, 29-32 years. The task required the monkey to free a ring-shaped Lifesaver candy by moving it along a metal rod which was bent into "routes" of varying complexity. The first stage consisted of 12 days of preliminary training on straight rods with four different orientations (right/left/up/toward). The second stage consisted of 12 'easy' patterns (1-3 90° bends), which were randomly presented three times each during a six day period. The final stage used 12 'difficult' patterns (2-5 90° bends) presented in the same fashion. A limit of 45 sec and 120 sec was allotted for solving each easy pattern and each difficult pattern, respectively. No significant differences among age groups were found in the fastest time to solve the pretest stage or the easy patterns ($F(3,14) = 2.97$; $F(3,14) = 2.5$, respectively). However, the four age groups differed significantly in the fastest time to solve the difficult patterns ($F(3,14) = 3.52$, $p<.05$). Paired comparisons revealed that Groups II, III, and IV required significantly more time to solve the difficult patterns than did Group I, and Group IV also performed significantly worse than Group II, indicating a progressive increase in the time to solve the pattern with increasing age. Because there were no group differences on the pretest and easy patterns, the deficits found among the older groups on the difficult patterns are less likely to reflect motor impairment than impairment in visuospatial orientation. The results of this investigation together with those of our previous studies suggest that in nonhuman primates multiple neural systems are vulnerable to increasing age.

452.4 QUANTIFICATION OF CEREBRAL VENTRICULAR VOLUME USING MAGNETIC RESONANCE IMAGING (MRI) IN THIRTY-NINE HUMAN SUBJECTS WITHOUT APPARENT PATHOLOGY. G.D. Cramer*, D.J. Allen+, L.J.A. DiDio*++, W. Potvin*++ and R. Brinker*++. Department of Anatomy, National College of Chiropractic, Lombard, IL 60148, +College of Health Science, Georgia State University, Atlanta, GA 30303 and ++Departments of Anatomy and Radiology, Medical College of Ohio, Toledo, OH 43699.

Comparative assessment of the accuracy of computerized tomography (CT) and MRI was previously performed and presented, showing MRI to be more accurate than CT in determining the volume of phantom preparations of the encephalic ventricles (Cramer, G.D. et al. Society for Neuroscience, Abstract, 1986). Utilizing the same methods as those of the previous study, the present study evaluated ventricular volume from the MRI scans of 39 subjects found to have no apparent pathology. Values for all four ventricles and the mesencephalic aqueduct were obtained for the 15 males and 24 females who made up the 39 subjects. The mean, standard deviation, and range of values were obtained for each ventricle. The average volume of the encephalic ventricles was found to be 17.2 cm^3. This was in close agreement with previously published volumes obtained from ventricular casts and from CT data. The volumes of the ventricles were found to increase slightly, yet significantly, with age. This, too, was in agreement with previously published CT data.

The average volume of the encephalic ventricles was found to be slightly greater in the female group than in the male group. The left lateral ventricle was found to be slightly larger than the right in females, and the right lateral ventricle was found to be slightly larger than the left in males. However, the difference was found to be insignificant statistically.

Volumes for each of the individual ventricles were obtained, and it was found that the average volume of the fourth ventricle was .7 cm^3. The average volume for the mesencephalic aqueduct was .1 cm^3; for the third ventricle, .7 cm^3; for the right lateral ventricle, 7.9 cm^3; for the left lateral ventricle, 7.9 cm^3. Search of the literature revealed this to be the first time volumes were obtained for the mesencephalic aqueduct in the living. It is hoped that this study will serve as a basis for future investigations of ventricular volumes in subjects with pathologically enlarged ventricles. The methods used to quantify ventricular volume could also be used to evaluate other neuroanatomical structures such as the basal ganglia. Supported in part by GSU Research Grant No. 87-024.

452.5 PERFORMANCE OF HEALTHY ELDERLY AND YOUNG ADULTS ON TWO PRIMING TASKS. H.P. Davis and A. Cohen* Dept. Psychology, Univ. Colorado, Colorado Springs, CO 80933.

A distinction between procedural memory and declarative memory has been extensively examined in amnesias of various etiologies, and more recently as a function of aging. Procedural memory refers to a domain of memory that is acquired through performance with implicit memory representation. Procedural tasks would include many tests of motor skills, perceptual skills, and cognitive skills. Declarative memory is explicit and refers to memory for such things as facts, personal and temporal episodes and would include tasks that require the recall or recognition of word lists. Some amnesics show normal acquisition and retention of procedural skills, and yet have markedly impaired declarative memory.

Studies using tasks that examine priming, a phenomenon thought to fall within the domain of procedural memory, have extended investigation of the procedural-declarative distinction to the elderly. Priming refers to a process whereby previous exposure to a stimulus affects subsequent processing of the same stimulus. Elderly (Mean Age = 80) and young (Mean Age = 22) adults matched on Full Scale IQ and years of education were administered three frontal lobe tests, word recall tests, word recognition tests, a word stem completion priming test, and a homophone priming test. For the word stem priming task, subjects were presented a list of words and instructed to rate each word on how well they like it. Later subjects were presented three letter stems and asked to complete the stem with the first word that came to mind. For the homophone priming task, subjects were given an objective test with the lower frequency member of homophone pairs embedded in the questions. Later subjects were given a verbal spelling test with homophones embedded in the test. Elderly were significantly impaired on frontal lobe tests and recall tests as compared to young adults. No difference in recognition performance was detected. Both young and elderly subjects showed significant priming effects on the word stem completion priming test ($p<.001$). However, young subjects showed significantly greater priming than the elderly ($p<.01$). Neither elderly or young subjects demonstrated significant priming on the homophone priming task.

The performance of the elderly on priming tasks is likely to vary as a function of instructions, task requirements, and the age of the elderly subject population. We have previously shown that elderly are impaired on other procedural tasks; a perceptual-cognitive task and a cognitive task. Thus, as compared to amnesics, the elderly demonstrate qualitatively different performance on procedural tasks.

452.6 PRESERVATION OF CIRCADIAN RHYTHMS OF ACTIVITY IN HEALTHY ELDERLY HUMANS. H. R. Lieberman, J. J. Wurtman* and M. H. Teicher. Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

The measurement of daily patterns of activity provides a non-invasive and unobtrusive behavioral measure of an organism's response to the external environment. We continuously monitored, for four days, patterns of activity of 77 healthy adult humans using a small, wrist-mounted, ambulatory activity meter. This device counted total number of above-threshold movements of an individual in 15-minute blocks of time and recorded the results in individual memory locations for later retrieval. Subjects were 15 young males (21-35), 18 young females (19-33), 20 elderly males (65-94), and 24 elderly females (65-85). During the study they resided at a clinical research center but were permitted to briefly leave on several occasions per day. They ate at standard pre-set times and were free to rise or retire whenever they wished. In this structured, institutional environment substantial differences in the absolute levels and patterns of daily rest and activity across age groups were observed. The elderly subjects were significantly more active than the young subjects ($p<.001$, overall) during both day (07:00-23:00 h) and night (23:00-07:00 h) periods ($p<.005$). Consistent with their increased levels of activity, the healthy elderly subjects reported feeling more alert and less sleepy and fatigued than the young volunteers, as measured by self-report mood questionnaires.

To characterize the circadian patterns of activity a non-linear cosinor analysis was performed on each individual's data. The age groups differed on several circadian parameters ($p<.0001$). The acrophase (peak of a sinusoid fitted to the activity rhythms) of the elderly group occurred at 13:29 $\pm$ 00:09 h (mean $\pm$ sem), significantly earlier than in the young group (15:15 $\pm$ 00:11 h). The amplitude and the mesor (mean level) of the rhythms were both greater in the elderly group.

It is uncertain whether the between-group differences observed reflect changes in behavior that occur as a consequence of the aging process, previously-established differences in the life styles of the different populations studied, some bias in subject recruitment, or some other factor. Regardless of the explanation, it is apparent that the individuals we recruited from these two age groups exhibited substantial differences in total activity and circadian rhythms of activity when living in a standard environment for several days. Although characteristics of circadian rhythms differed between the two age groups, our initial analyses suggest that there was no deterioration of this rhythmicity in this very healthy elderly group.

452.7 REGIONAL CEREBRAL BLOOD FLOW AND CLINICAL CORRELATES OF DEGENERATION IN EARLY ALZHEIMER'S DISEASE

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Previous studies of regional Cerebral Blood Flow (rCBF) in Alzheimer's disease (AD) have documented cortical perfusion deficits in long duration AD patients. Accurate differential diagnosis and correlation with neuropathological findings have been demonstated in these samples (Risberg and Gustafson, 1983). In this laboratory, accurate differential diagnosis has been shown in the early stages of disease progression. The purpose of the current study was to examine the sensitivity of rCBF to disease progression in short duration AD patients and to evaluate the relation between functional decline and rCBF.

Methods Ten AD patients were referred by a neurologist and given the diagnosis of AD based upon comprehensive examination. Disease progression was quantified by indices of cognitive function (Modified Mini-Mental Status Test,mMMS, means=33.30 +15.69/ 27.6+17.48, first and second visit, respectively), impairment of activities of daily living (Blessed Dementia Rating Scale,BDRS, mean=8.10+2.68/ 11.15+ 4.87) and estimated disease duration (mean=2.6+1.80/ 3.6+ 1.63).

rCBF was measured by the 133-Xe inhalation technique, with 16 detectors over each cerebral hemisphere. The data presented in this report were derived with a four-compartment model (Prohovnik et. al., 1983) and are presented in terms of the Initial Slope Index (ISI, means= 42.14+4.89/ 37.07+8.68), a measure representing primarily grey matter flow. Subjects were evaluated on two separate occasions (mean follow-up interval=1.0 years+0.447) The studies in this report were conducted with the subject at rest, with eyes closed and ears unoccluded.

Results Repeated measures analysis of variance revealed significant declines in mMMS (F=10.82, p=.010),BDRS (F=7.88, p=.021) and global grey matter flow (F=9.66, p=.015) at follow-up evaluation. Decline in grey matter flow was significantly correlated with decline in mMMS (r=.72, p,<.05). Significant correlations between declines in mMMS and regional flow values were found in bilateral inferior frontal and parieto-temporal areas . These regions show the greatest degree of deficit when AD patients are compared to age matched normal controls (Prohovnik, et.al., 1986). Change in BDRS and duration were not significantly correlated with flow. There were no significant intercorrelations within the severity indices.

These results demonstrate significant deterioration in the early stages of AD at short-term follow-up and support the sensitivity of rCBF in documenting disease progression.

452.8 ALZHEIMER'S DISEASE: CEREBRAL METABOLIC ABNORMALITIES COINCIDE WITH EARLY MEMORY DEFICITS

W.H. Riege, E.J. Metter*, D.E. Kuhl, A.B. Lanto*, G.W. Small*, D.G.Fujikawa, J.C. Mazziotta, D.A. Dorsey*, and A. Maltese*. VA Medical Center Sepulveda and UCLA School of Medicine, Los Angeles, CA. 90024

Studies using (F18)-fluorodeoxyglucose (FDG) with positron emission tomography (PET) have documented a characteristic pattern of lowered cerebral metabolic rate (CMRGlc) in cortical gray regions of probable Alzheimer's disease (pAD) patients (Kuhl et al., J.Cer.Blood Flow & Met.,5, 169, 1985). Parietal and temporal CMRGlc are reduced relative to occipital or sub-cortical regional rates. The parietal/caudate-thalamic ratio (P/CT), in particular, distinguished between controls and early pAD. In some patients with mild pAD the right-left parietal (R-Lpar) metabolic asymmetries preceded the disproportionate language problems seen in moderate AD patients (Haxby et al., Arch.Neurol., 43, 882, 1986). However, verbal memory difficulties may occur even before mild CMRGlc abnormalities do, so that their correlation with P/CT can be diagnostic for early pAD.

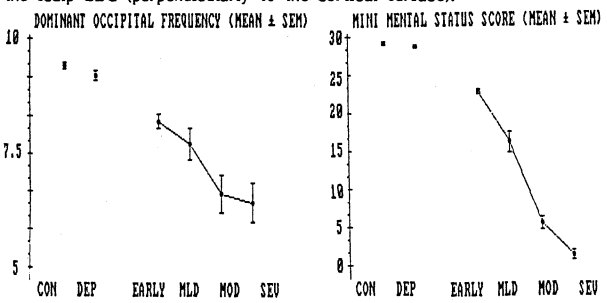
We studied 26 persons (age=66.8+/-5.4 years), complaining of increasing memory difficulties, with both FDG-PET brain scan (NeuroECAT, resolution 1.2 cm FWHM) and an 18-test memory set. After neuropsychological screening, they were categorized by the Clinical Dementia (CDR) and NINCDS rating into mild possible AD (CDR 0.5, n=14) and moderate pAD (CDR 1.0, n=12); all other diagnoses of possible dementia had been excluded. On sensitive tests of visual, auditory, tactual, and verbal memory using signal detection measures relatively free of guessing bias, both pAD groups were impaired ($F = 8.71; 16,76; p< 0.0001$), compared with 21 normal controls (age = 65.3 +/- 6.7 years). The mild pAD performed better than moderate pAD on verbal recall and timed learning tests ($p< 0.03$), at the time of the first but not at the second brain scan 1 year later. Their regional CMRGlc's did not differ from that of controls but the R-Lpar ratio deviated by >21% and the P/CT ratio by 25% ($t= 7.81, p< 0.0001$) from controls. At the repeat scan 1 year later, the mild (possible) AD had deteriorated to probable AD and had lower scores on visual and verbal memory tests than at first scan but decreased their P/CT only nonsignificantly; controls showed no deviations. At the first scan, the hypometabolism expressed in the P/CT ($r>0.50$) as well as the R-Lpar asymmetry ratio correlated with visual memory difficulties in the mild pAD indicating that memory deficits coincide with subtle brain metabolic abnormalities already early in pAD.

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452.9 A POSSIBLE EEG BIOMARKER FOR ALZHEIMER DISEASE: DOMINANT OCCIPITAL FREQUENCY (DOF)

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DOF (or alpha rhythm) is a predominating rhythmic EEG activity seen in occipital or other scalp regions. DOF is considered to be generated by EPSPs, IPSPs or other membrane voltage fluctuations in underlying neuronal membranes(1). DOF is known to slow in frequency with normal aging, possible as a result of age reductions in dendritic spines. Further decreases occur in Alzheimer's Disease (AD). Here we report that DOF slows in AD in parallel with the severity of AD. Subject groups were: 85 controls (CDN), 42 major depressives (DEP)(DSMIII), 46 early AD patients (EARLY)(Possible or Probable, NINCDS), and 16 mild (MLD), 16 moderate (MOD) and 10 severe (SEV) stage AD patients. Groups were matched on health and demographics(2). Ten hours continuous all night sleep-wake EEG recordings were obtained in healthy, drug free subjects (aged>55). Blind ratings of DOF were based on ten or more 1 sec frequency counts obtained when activity was most rhythmic, during quiet wakefulness. Figure 1 shows group means + SEM for DOF and for a measure of AD severity, the Mini Mental Status Score. The EARLY AD group had significantly lower DOF scores compared to the CON ($F=61.1, p<.001$) and DEP ($F=23.1, p<.001$) groups. DOF continued to decline with increasing severity of AD. The data are consistent with the hypothesis that DOF may index AD related cortical dendritic degeneration, particularly the loss of functioning, cortical neurites oriented such that their potential fields are detectable in the scalp EEG (perpendicularly to the cortical surface).



1Lopes da Silva, in "Electroencephalography", 1982, Urban,pp. 15-26.
2Prinz, et al., Neurobiol. Aging, 3:4, 361-370, 1982.

452.10 DOMINANT OCCIPITAL FREQUENCY: A POSSIBLE USEFUL BIOMARKER OF EARLY STAGE ALZHEIMER'S DISEASE.

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Several studies have reported the potential usefulness of quantifications of cortical slowing to serve as a biomarker of Alzheimer's Disease (AD), all have involved AD patients with significant cognitive impairment. Here we report on the usefulness of Dominant Occipital Frequency (DOF) to distinguish early stage AD patients. 85 control (C) subjects (67.8+7.1 years, 46% male) and 46 AD subjects (70.4+7.5 years, 44% male) were studied. Control subjects were further divided into three groups based on memory complaint; non-complainers (NC, N=29), complainers without validation (NVC, N=24) and complainers whose problem was validated by a significant other (VC, N=32). AD patients were divided by NINCDS criteria into Possible AD (POSS, AD N = 16) and Probable AD (PROB AD, N=30) groups. DOF was quantified by 10 or more one second frequency counts from the quiet wake EEG. Subjects were administered a number of cognitive and mental status tests including the Mini-Mental State (MMS) and Rivermead Behavioral Memory Test (RBMT). DOF and all cognitive test scores declined in a parallel hierarchical manner across these five groups with the NC group having highest scores and PROB AD group having lowest scores.

	NC	NVC	VC	POSS AD	PROB AD
DOF	9.6±.6	9.4±.5	9.3±.6	8.8±.7	7.9±1.0
MMS	29.5±.8	29.7±.5	28.3±1.7	24.9±1.5	21.7±2.8
RBMT	104.3±9.2	104.6±9.8	94.7±17.7	61.0±14.9	51.5±15.8

Discriminant analyses examined this hierarchical progression of DOF for its ability to classify subjects into groups. DOF yielded an overall classification rate of 80% (72% of AD and 85% of C). DOF did not perform well in classifying NC versus NVC groups and NC + NVC versus VC groups (57% and 56% overall correct classification, respectively). DOF provided better discrimination of POSS AD versus PROB AD groups (67% correct), good discrimination of VC versus POSS and PROB AD groups (74%), and excellent discrimination of NC + NVC versus POSS + PROB AD groups (84%). These results indicate that DOF may be a useful biomarker of AD even in the early stage of the disease. As followup assessments improve the accuracy of AD diagnosis the ability of DOF to correctly discriminate AD patients from controls may improve.

452.11 LACK OF INTRA-PATIENT CONSISTENCY IN RATE OF PROGRESSION IN ALZHEIMER'S DISEASE. T. John Rosen[1,2*], John H. Growdon[2,3], and Suzanne Corkin[1,2]. 1. Dept. Brain and Cognitive Sciences, Mass. Institute Technology, Cambridge, MA 02139; 2. Clinical Research Center, Mass. Institute Technology, Cambridge, MA 02139; 3. Dept. Neurology, Mass. General Hospital, Boston, MA 02114.

Alzheimer's disease (AD) is a progressive, dementing disorder that compromises a variety of cognitive, sensory, and motor functions. Researchers and clinicians are impressed by the substantial differences between patients in the disease's symptoms and rate of progression. What is not known is whether individual patients' rates of change early in the disease are related to their rates of change later on. We therefore studied intra-patient consistency in rate of deterioration among 25 patients throughout an average of 2.31 years; we observed no correlation whatsoever.

All patients were diagnosed by standard procedures as having probable AD; all were seen at least four times in the memory disorders clinic of a general hospital. At each visit, patients received the Blessed Dementia Scale (Blessed, Tomlinson, & Roth, 1968), a standard measure of dementia severity that included subscales for activities of daily living and for memory and information. Scores of 0-2 were considered normal; the maximum total score was 65. The mean total Blessed score at the initial visit was 18.3 (SD = 8.2). The consistency of deterioration was determined by analyses of rate of change (in points per year) from Visits 1 to 2 (mean interval = .798 years) and from Visits 3 to 4 (mean interval = .711 years). Correlations between rate of change from Visits 1 to 2 with that from Visits 3 to 4 did not approach significance for either Blessed subscale or for total score (all p values $\geq .50$).

This finding demonstrates that individual AD patients are not consistent in their rate of decline during the interval studied. The results suggest caution on the part of physicians who extrapolate later rate of progression from earlier observations.

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452.12 THE PROGRESSION OF SENILE DEMENTIA OF THE ALZHEIMER TYPE IN PSYCHOTIC VERSUS NON-PSYCHOTIC SUBJECTS. E.H. Rubin and W.C. Drevets*. Dept. of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110

As part of the Alzheimer Disease Research Center and Memory and Healthy Aging project at Washington University Medical School, we have been longitudinally following 58 controls and 43 subjects with initially mild senile dementia of the Alzheimer type (SDAT; clinical dementia rating 1 (CDR1)) for over 5 years. Diagnosis and severity rating were based on a physician administered, 90 minute semi-structured protocol involving the subject and a collateral source (Berg et al., J. Neurol. Neurosurg. Psychiatry, 45:962-8, 1982; Br. J. Psychiatry, 145:339, 1984; Hughes et al., Br. J. Psychiatry 140:566-72, 1982). Subjects with a history of potentially confounding medical or psychiatric illnesses including depression, psychoses, or alcoholism were excluded. Our population, therefore, is highly selective for SDAT in otherwise healthy individuals.

Twenty-two of the 43 subjects (51%) developed psychotic symptoms during the course of the illness. Twenty-six percent became psychotic when the illness was in a mild stage. By the time a moderate stage of SDAT (CDR2) had been reached, 42% demonstrated psychotic symptoms. In the 26 subjects who reached a severe stage of SDAT (CDR3), 54% had developed psychotic symptoms during the course of their illness. Delusions were more common than hallucinations.

Subjects were evaluated approximately every 16 months throughout the study. When compared to those who remained free of psychotic symptoms and were not lost to follow-up (n=15), subjects with early psychotic symptoms (n=10) deteriorated more rapidly in terms of cognitive status as assessed by the CDR, the Blessed Dementia Scale and the Short Portable Mental Status Questionnaire of Pfeiffer. On the other hand, by the end of the 66 month follow-up, 8 of the 10 subjects with early psychosis were still alive versus 6 of the 15 non-psychotic subjects ($.05<p<1.0$). Therefore, early psychosis in SDAT correlates with more rapid cognitive deterioration but not increased mortality.

TRANSMITTERS AND RECEPTORS IN DISEASE V

453.1 ACUTE, MASSIVE STRIATAL DOPAMINE RELEASE IS ASSOCIATED WITH STRIATAL VULNERABILITY TO TRANSIENT ISCHEMIA IN THE RAT. M.Y.-T. Globus, M.D. Ginsberg, R. Busto, W.D. Dietrich, E. Martinez, and I. Valdes. Cerebral Vascular Disease Research Center, University of Miami, School of Medicine, Miami, FL, 33101.

Neurotransmitter release is believed to play a major role in mediating ischemic damage in selectively vulnerable brain regions. Small- to medium-sized striatal cells are among neurons known to be selectively susceptible. The striatum, which receives abundant glutamatergic input from the neocortex, is also richly innervated by the nigrostriatal dopaminergic projections. In this study we evaluated the effect of dopamine (DA) depletion on the extracellular release of striatal glutamate (Glu) and DA during ischemia and recirculation, and correlated that with postischemic histopathological changes. Two weeks after unilateral SN lesion, a microdialysis probe was sterotaxtically implanted into the ipsilateral striatum and subsequently perfused with Ringer's solution at a flow rate of 2 ul/min. After a stabilization period of 1.5 h, rats were subjected to 20 min of ischemia by 4-vessel occlusion combined with systemic hypotension. Samples of the perfusate, representing extracellular fluid, were collected at 10 min intervals, 30 min before ischemia; during ischemia; and during the first 30 min and at the end of 1 h and 2 h of recirculation. The samples were analyzed for DA by HPLC-EC and for Glu by enzymatic assay. A different group of SN-lesioned rats (n = 8), to be evaluated for histology, were perfusion-fixed after 3 days of recirculation, and quantitative counting of ischemic neurons was carried out in four 500 x 500 um microscopic fields within the dorsolateral striatum. In the control non-lesioned rats (n = 5), a significant increase in striatal DA (baseline levels: 4 to 65 pmol/ml, ischemic levels: 950 to 2300 pmol/ml) and Glu (baseline levels: 0.6 to 11.5 nmol/ml, ischemic levels: 1.34 to 32.5 nmol/ml) was detected in the perfusate within 10 minutes of ischemia. This increased release persisted during the 20 min of ischemia and the levels gradually returned to baseline values by 30 min of recirculation. In SN-lesioned rats (n = 5) no changes were found in extracellular levels of DA during ischemia in the ipsilateral striatum, although significant increases in glutamate were measured. In non-lesioned rats, profound and highly reproducible histopathological alterations of ischemic injury, similar in degree on the two sides, were found throughout the striatum. In contrast, ischemic striatal damage was markedly attenuated by SN-lesion. While on the non-lesioned side, there were 55 $\pm$ 6 ischemic neurons per high-power field (mean $\pm$ S.D.), on the DA-depleted side only 17 $\pm$ 24 ischemic neurons were found ($P < 0.01$ by paired t-test). Our results indicate that DA release during ischemia is a prerequisite for the development of ischemic striatal damage. (Supported by USPHS Grants NS05820 and NS22603.)

453.2 PHARMACOLOGICAL CYTOPROTECTION OF CORTICAL CATECHOLAMINES AFTER EXPERIMENTAL EMBOLIC STROKE. J.C. de la Torre, T.R. Fortin,* M.T. Richard*. Div. of Neurosurgery, Univ. Ottawa and Ottawa General Hosp., Ottawa, Ont. K1H 8M5.

The biochemical cascade that follows embolic stroke has been causally linked to numerous sub-systems. Among the sub-systems that have been suggested as the triggering cause for the pathophysiology seen after stroke is (a) calcium release, (b) hydroxyl radical formation, (c) prostaglandin changes and (d) opioid receptor activation. Studies have shown that when cerebral blood flow (CBF) falls to 20 ml/100 g tissue/min., neuroelectric activity is significantly reduced in brain. If CBF is further lowered to 12-15 ml, neuroelectric activity may be abolished. A CBF of 6 ml or below generally leads to cell membrane ionic imbalance and eventual neuronal death. The duration and extent of reduced CBF may therefore critically determine the clinical outcome of occlusive stroke (Astrup, J., J Neurosurg 56:482 (1982); Trauppe, H. et al, J CBF Metab 1: (suppl. 1) S198 (1981). In the present experiment, we investigated the effect of drugs that affect the sub-systems related to calcium, hydroxyl radicals, prostaglandins and opioid receptors to determine whether they could modify an experimentally-induced vascular occlusion in brain. Cats were randomly divided into 6 groups and subjected to middle cerebral artery occlusion (MCAO) via a transorbital approach. After 1 or 4 hours, each group received the following compounds: (i) saline (S); (ii) nalaxone (NX) 2 mg/kg, (iii) nimodipine (NM) 1 µg/kg; (iv) prostacyclin (PGI_2) 0.2 mg/Kg/min x 25 min.; (v) dimethyl sulfoxide (DS) 1.5 g/Kg, 40% solution. Following these results, an additional group was added to test the outcome of PGI_2 + DS. At various time points, local cerebral blood flow (lCBF) was measured using micro-electrodes in cortical tissue surrounding the MCAO. Histologic examination and grading of cortical catecholamines and neuronal tissue was done after MCAO.

Our results indicate that only PGI_2 and DS were able to modify lCBF reductions after MCAO. The combination of PGI_2 and DS in addition to preventing severe CBF fall, appeared to be cytoprotective of the cortical ischemic tissue as demonstrated by sparing of catecholamine-containing varicosities in cortex following the MCAO insult.

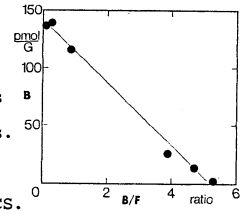
Supported by the Heart and Stroke Foundation of Ontario.

453.3 **IN VIVO MEASUREMENTS OF RAT BRAIN Bmax AND KD OF (^{125}I)HEAT, AN α-1-ADRENOCEPTOR ANTAGONIST.**
S. Dyve*, A. Gjedde*, M. Diksic*, A. Sherwin*, and A. Hakim*. (Spon: L. Wolfe). Montreal Neurological Institute and Hospital, Montreal, Quebec, Canada

Studies in rat brain have shown (125)Iodo-2-(beta-(4-hydroxyphenyl)-ethylaminomethyl tetralone, to be a high affinity α-1-adrenoceptor antagonist, binding to discrete areas of brain. This study aimed to characterize the blood-brain barrier's (BBB) permeability to the ligand, volume of distribution and kinetics of regional uptake.

1) BBB permeability and volume of distribution: 30-40 μCi of ligand (SA 2200 Ci/mmol) were injected i.v. Rats were decapitated at various times following injection, up to one hour. Blood samples were taken continuously. Autoradiograms were prepared from 20 μM sections. 2) Kd and Bmax: 38 μCi (^{125}I)HEAT and from 0.5-500.0 nmol HEAT were injected i.v. and circulated for 1 hr. Blood samples were taken at the time of decapitation. Autoradiograms were prepared as above.
RESULTS: 1) Regional activity of (^{125}I)HEAT was plotted against the normalized plasma time-integral. The BBB permeability (K1) averaged 0.3%+0.04 (SE) ml/g/min. The cerebellar cortex showed no binding and the steady-state volume of distribution (partition coefficient) averaged 2.2%+0.03 ml/g. Binding regions included the olfactory bulb, frontal-parietal cortex, thalamus, medial geniculate and pineal gland. In these regions, the equilibrium volume of distribution averaged 5.4%+0.6 (S.E.M.) indicating a Bmax/Kd ratio (receptor potential) of 4.4. 2) The affinity, Kd, and receptor density, Bmax, were calculated by Scatchard analysis (n=6) in various regions. A Scatchard plot representing the ventr. post. thalamic nucleus is shown; Bmax, 141(4) pmol/g; Kd 27(1) nmol/l.

(^{125})HEAT crosses the BBB, binding in discrete regions which represent functional organization. Earlier studies in rats that had received electroconvulsive shocks (ECS) have shown that the α-1 receptor density increases 24 hrs. after the last ECS. In vivo experiments are planned to evaluate receptor density changes at various times after ECS.



453.4 **RAPID REDUCTION OF GERBIL FOREBRAIN ALPHA$_1$-ADRENERGIC RECEPTORS DURING CAROTID OCCLUSION.**
K. Nishino* and J.N. Davis. V.A. Medical Center and Depts. of Medicine (Neurology) and Pharmacology, Duke Univ., Durham, NC 27705.

In working with brain alpha$_1$-adrenergic receptor binding, we noticed a reduction in receptors in animals subjected to mild ischemia before sacrifice. In order to characterize this reduction, we subjected Mongolian gerbils (55-70 gms) to 5 or 10 minutes of occlusion under 0.5 - 1.0% halothane anesthesia. After occlusion, some animals were allowed to survive for an hour. Most animals were sacrificied by decapitation at the end of the occlusion period and their forebrains rapidly dissected and frozen on powdered dry ice. Controls were subjected to sham operation and anesthesia. Membrane homogenates were prepared from frozen forebrains and [^{3}H]-prazosin binding measured at equilibrium using rapid filtration and liquid scintillation counting.

Less [^{3}H]-prazosin binding sites were present in gerbils exposed to 10 minutes of ischemia (57.0 ± 0.1 fmol/mg) compared to controls (117.7 ± 0.2), but the affinity of the binding sites for [^{3}H]-prazosin was unchanged (1.7 nM). Decreases in [^{3}H]-prazosin binding were present after 5 minutes of occlusion, but were maximal after 10 minutes and persisted at 1 hour of reflow.

In another experiment, alpha$_1$-adrenergic binding was compared to beta adrenergic and muscarinic cholinergic receptor binding in the same membranes. Binding was reduced in membranes from animals after 10 minutes of carotid occlusion compared to controls by 61% ([^{3}H]-prazosin), 25% (beta$_1$, [^{125}I]-cyanopindolol + ICI 118,551), 33% (beta$_2$, [^{125}I]-cyanopindolol + ICI 89,406), and 18% ([^{3}H]-QNB). Unexpectedly protein content of the ischemic membranes increased by 20%.

These experiments show that there is a rapid loss of alpha$_1$-adrenergic receptors in ischemic forebrain and that this loss is greater than decreases in at least some other membrane receptors. Although the underlying mechanism and biological significance of this observation are not yet clear, we speculate that the change could play an important role in the chain of events that leads to selective neuronal damage after transient ischemia.

(Supported by the V.A. and NS 06233)

453.5 RIGHT CAROTID OCCLUSION ALTERS DIHYDROPYRIDINE CALCIUM ANTAGONIST BINDING IN VARIOUS RAT BRAIN AREAS.
M.S. Magnoni*, S. Govoni, F. Battaini^^, F.Rossi*^ and M. Trabucchi^^. Institute of Pharmacological Sciences, University of Milan, ^ Istituto Scientifico Sanatrix, Venafro and ^^Chair of Toxicology, 2nd University of Rome, Italy.

Increasing evidence suggests the role of calcium ions in the pathophysiology of the ischemic brain damage. In particular, the interruption of cerebral blood flow results in a chain of events, sustained by the increased influx of calcium ions in the ischemic cells, that ultimately lead to neuronal necrosis.
The major mechanisms allowing calcium entry from the extracellular compartment is the opening of voltage operated calcium channels (VDCC). Three different types of VDCC (T,N,L) have been identified in neurons, which differ for electrophysiological and pharmacological features. In particular, L-type is specifically sensitive to organic calcium antagonists of the dihydropyridine (DHP) class and may be studied at biochemical level using tritiated DHPs. In this line, we have explored the hypothesis that the characteristics of L-type calcium channels, may be modified by ischemia.
Cerebral ischemia was experimentally induced in rats by 1 h ligature of the right carotid. Tritiated PN 200-110 binding was measured in P2 membranes from hippocampus, cortex and striatum according to the method described by Supavilai and Karobath (J. Neural. Transmission 60:149,1984) with minor modifications.
The results show that mild ischemia induces a significant increase in the number of binding sites in the hippocampus ipsilateral to carotid occlusion (left (non occluded): 220 ±10 f.moles/mg prot.; right (occluded): 292±23* f.moles/mg prot.; *P < 0.05), accompanied with an increase in Kd values. In striatum and cortex no changes either of Bmax or of Kd have been detected. This event may be related to the increase of calcium ion influx into the cells in ischemic conditions. It is important to note that this effect is selective, at least at early time, for the hippocampus, a cerebral area particularly vulnerable to ischemic insults and rich in calcium channels.
The ischemia-induced modifications of PN 200-110 binding characteristics suggest the involvement of L-type calcium channels in the alterations of neuronal excitability and calcium movements observed during ischemia.

453.6 INVOLVEMENT OF ACETYCHOLINE RECEPTOR AND NON-CHOLINERGIC ANTIGENS IN HUMAN MYASTHENIA GRAVIS. ISOLATION OF cDNA CLONES FROM HUMAN MUSCLE AND THYMUS.
Mirta Mihovilovic*, John Gilbert* and Allen D. Roses. Division of Neurology, Duke Univ. Med. Ctr., Durham, NC 27710.

Myasthenia Gravis (MG) patients produce auto-antibodies directed against the nicotinic acetylcholine receptor (AcChR) and other poorly characterized non-cholinergic antigens expressed in skeletal muscle. Thymic hypertrophy and thymoma are commonly associated with MG. MG antibodies recognize antigens shared by muscle and thymus (including a putative thymic AcChR) suggesting that thymic antigens may be involved in the pathogenesis of MG.

In order to study the non-cholinergic antigens involved in the MG autoimmune response, we have constructed a λgt11 cDNA expression library from adult human skeletal muscle. This library was screened with MG sera reactive toward a series of muscle antigens (as determined by Western blotting of human muscle extracts and detection of immunoreactive material in these blots by MG sera). We report the isolation of four muscle-specific clones (1.3 - 1.9 Kb) that do not react with normal human sera but show reactivity toward MG sera lacking anti-AcChR activity or activity against denatured AcChR subunits.

To investigate the role of thymic antigens in MG we have focused our attention on the well-characterized AchR. The thymic expression of mRNA species that code for a putative cholinergic antigens has been investigated. Probing Northern blots with RNA transcripts from a subclone, (internal PstI-HincII fragment) derived from the clone pMARα15 that codes from the α subunit of the AcChR of the mouse cell line BC3H-1 (Boulter, J. et al., J. of Neurosciences 5, 2545-2552, 1985), we have detected a thymic mRNA species with a length of approximately 3.0 Kb using high stringency conditions. The muscle mRNA species detected by the probe under identical conditions shows a length of approximately 2.2 Kb. Employing the same probe we have isolated from a human thymus library two cDNA clones each one carrying an approximately 3.0 Kb insert. The relationship between these clones and their muscle counterparts is currently under investigation.

453.7 EFFECTS OF NICOTINE ON PLATELET UPTAKE OF SEROTONIN IN-VIVO AND IN-VITRO. T.A. Kent*, P. Cinciripini*, J. Campbell*, R. Gevedon* (SPON: R.R. McKendall). Depts. of Neurology, Psychiatry, and Pharmacology, Univ. of Texas Medical Branch, Galveston, TX, and (JC) Dept. of Psychiatry, Univ. of Kansas Medical CTR, KC KS.

Blood platelet uptake of serotonin (5HT-uptake) has been of interest as a biological marker of neurotransmitter abnormality in psychiatric and neurological disorders. Because of the high incidence of smoking in these patients, the effects on 5HT-uptake need to be clarified. Nicotine (NIC) has been shown to enhance the stimulated release of 5HT in animals after chronic administration. We were interested in whether these effects were reflected in human subjects. Three studies were performed: (1) 5HT-uptake in cigarette smokers versus age-matched controls, (2) 5HT-uptake in smokers abstinent overnight before and after high (1 mg NIC) or low (0.5 mg NIC) dose cigarettes, (3) the effects of varying doses of NIC on 5HT-uptake in-vitro on platelets from non-smokers, abstinent smokers, and sated smokers. 5HT-uptake was determined after a 3 minute incubation with 1 X 10^{-7} ^{3}H-5HT (Kent, et al, J Alc Studies, 1985). As in other studies, 5HT-uptake showed a seasonal variability, decreasing significantly during winter months. Seasonally adjusted 5HT-uptake was approximately 13% higher in sated smokers ($p>.05$). However, 5HT-uptake was significantly lower by 25% in abstinent smokers. Uptake subsequently increased in 11 out of 14 cases 5 minutes after a high dose cigarette, but in only 4 out 7 cases with the low dose cigarette. NIC in-vitro decreased 5HT-uptake dose-dependently (a 25% decrease at 1 X 10^{-6} M) in platelets of non-smokers. However, in platelets from abstinent smokers, there was no consistent effect, suggesting that chronic NIC exposure altered the responsiveness of 5HT-uptake to NIC. These results demonstrate that platelet 5HT-uptake is not altered in smokers when examined during the normal smoking cycle. However, uptake may be lower in abstinent subjects (e.g. if studied upon arising without given access to a cigarette). This effect on 5HT-uptake may also be reflected in measurement of other parameters of 5HT function such as by hormonal probes. These results may also relate to behavioral or addictive effects of NIC.

453.8 EFFECTS OF BMAA AND BOAA ON KAINATE- AND QUISQUALATE-RECEPTOR BINDING IN ADULT MOUSE BRAIN. S.M. Ross and P.S. Spencer. Departments of Neuroscience, Neurology and Pathology, Institute of Neurotoxicology, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Beta-N-methylamino-L-alanine (BMAA) and beta-N-oxalylamino-L-alanine (BOAA) are chemically and neuropharmacologically related non-protein convulsant amino acids present in seeds of Cycas circinalis L. and Lathyrus sativus L., consumption of which has been linked to Guam amyotrophic lateral sclerosis (ALS) and lathyrism (a form of primary lateral sclerosis), respectively. Structural (BMAA) and/or functional (BOAA) changes of giant Betz cells, features of both human degenerative disorders, dominate the primate motor-system diseases induced by repeated subconvulsive doses of these amino acid analogues. Large single doses of BOAA or BMAA precipitate seizures and postsynaptic neuronal vacuolation (PSV) in mouse CNS tissue in situ and in vitro, respectively, excitotoxic actions that are markedly attenuated by glutamate-receptor antagonists. BOAA-induced PSV and behavioral alterations are blocked in a dose-dependent manner by cis-2,3-piperidine dicarboxylic acid, a quisqualate (QA)- and kainate (KA)-receptor antagonist, while those associated with BMAA are comparably modified by the selective NMDA-receptor antagonist, 2-amino-7-phosphonoheptanoic acid. The present study examined ^{3}H-KA-receptor binding (K_d = 14 nM; B_{max} = 0.38 pmol/mg protein) and ^{3}H-AMPA-receptor (i.e. QA-receptor) binding (K_d = 57 nM; B_{max} = 0.57 pmol/mg protein) in adult whole mouse brain membranes in the presence of BOAA, BMAA, or methylazooxymethanol (MAM), an oncogenic constituent of Cycas circinalis. MAM (1 mM) failed to modify ^{3}H-AMPA radioligand binding, and BMAA (0.5 mM) weakly displaced ^{3}H-KA and ^{3}H-AMPA binding (44% and 56% of controls, respectively). BOAA was approximately 700-fold more effective in displacing ^{3}H-AMPA binding than BMAA and only 45-fold more active than BMAA in displacing ^{3}H-KA binding. These results support and extend previous observations concerning the effects of BOAA and BMAA in behavioral and CNS tissue-culture paradigms. Whereas high concentrations of BMAA have little or no significant activity at QA and KA sites, BOAA has extensive interaction with the QA-receptor and may have some minor influence at the KA-receptor site. Thus, low levels of BOAA, in vivo, might initially exert excitotoxic potential through QA-preferring glutamate receptor sites, while higher levels would likely stimulate both QA- and KA-preferring receptor systems. BMAA, a non-dicarboxylic amino acid with delayed seizuregenic action, is likely to act indirectly via the NMDA-receptor. Supported by NS 19611 and a grant from the Muscular Dystrophy Association.

453.9 EFFECTS OF PYRAZINAMIDE AND TRYPTOPHAN ON CORTICAL QUINOLINIC ACID CONTENT IN RATS: AN ANIMAL MODEL OF GLUTARIC ACIDURIA TYPE I? M.P. Heyes* and S.P. Markey* (SPON: J. Johannessen). Lab. Neurophysiology and Lab. Clinical Science, NIMH, Bethesda MD 20892

Glutaric aciduria type I (GA-I) is an autosomal recessive neurodegenerative disorder of children associated with a deficiency in glutaryl-CoA (Gl-CoA) dehydrogenase. This enzyme catabolizes substrate derived from dietary tryptophan (TRP) that could othetwise be converted to the potent neurotoxin quinolinic acid (QUIN). Therefore, production of QUIN may be increased in GA-I and cause the neuropathology. To test this hypothesis, we have developed a gas chromatography/mass spectrometry assay for QUIN in brain. We observed, in rats, the effects of pyrazinamide (PYR), a drug that inhibits substrate entry into the Gl-CoA pathway. Neutralized perchloric acid extracts of brain, containing [^{18}O]-QUIN as the internal standard, were applied to Dowex 1X8 (formate). QUIN was eluted with 6N formic acid. QUIN and [^{18}O]-QUIN were derivatized to dihexafluoroisopropanol esters (mass 467 and 471, respectively) and negative chemical ionization was used to obtain a spectrum of masses m/z 467, 316 and 167 from QUIN and m/z 471, 320 and 167 from [^{18}O]-QUIN. Endogenous QUIN was quantified (in two cortical regions run in separate assays), by monitoring the ratio between m/z 467:471 and m/z 316:320. Systemic TRP (300 mg/kg) increased cortical QUIN; co-administration of PYR (300 mg/kg) potentiated this increase. In a separate study, systemic TRP, kynurenine (KYN), or 3 hydroxyanthranilic acid (3-OHAA) at doses of 300 mg/kg increased QUIN in cortex, striatum and thalamus.

CORTICAL QUIN (pmol/mg)					
SALINE	TRP	PYR	TRP+PYR	KYN	3-OHAA
0.2±0.1	1.5±0.7*				
1.9±0.4	6.7±1.1*	2.2±0.5	12.4±1.0**		
0.3±0.1	2.8±1.0*			1.0±0.2*	4.4±1.8*

n=8 or 10 per group; *$p<0.01$ vs. saline, **$p<0.01$ vs. TRP

We conclude that cortical QUIN is variable between different batches of rats. Further, brain QUIN is sensitive to the availability of TRP, KYN and 3-OHAA and is influenced by blockade of TRP catabolism through the Gl-CoA pathway by PYR. Rats treated with PYR may serve as an animal model for GA-I.

453.10 **ADENOSINE RECEPTOR-MEDIATED MODULATION OF SEIZURE ACTIVITY ORIGINATING IN THE PREPIRIFORM CORTEX.** P.H. Franklin, E.D.Tripp*, M. Fabrazzo*, K. Gale, T.F. Murray. Coll.of Pharmacy, Oregon State Univ., Corvallis, OR 97331; Dept. of Pharmacology, Georgetown Univ. School of Med., Washington, D.C. 20007

Although a great deal of evidence indicates a role for adenosine in the etiology or control of seizures, the neuronal circuitry subserving these actions is unknown. A discrete region- possibly of fundamental importance to epileptogenesis- exists within rat prepiriform cortex (PC) and has been referred to as Area Tempestas. Focal injections of bicuculline (BIC) into PC elicit bilateral motor seizures. We find that injections of 2-chloroadenosine (2CLA) into this site prior to BIC administration greatly reduce the severity of seizures observed. This effect of 2CLA was observed after a dose as low as 83 picomoles; at higher doses, some animals were completely protected.

In addition, we find that intraperitoneal injection of the adenosine receptor antagonist, theophylline (50mg/kg), increases the sensitivity to BIC induced seizures in the PC. Together, the proconvulsant effect of theophylline and the anticonvulsant effect of 2CLA suggest that the PC is one area where adenosine plays an important role in regulation of seizure activity.

Anticonvulsant effect of 2CLA against bicuculline induced seizures in the prepiriform cortex

TREATMENT	DISTRIBUTION OF SEIZURE SCORES* 0	1	2	3	4	5	MEAN SEIZURE SCORE
CONTROL					2	3	4.6
25ng 2CLA	2				1	1	2.8
CONTROL	1					2	3.33
50ng 2CLA	3						0.0
CONTROL	1					2	3.33
300ng 2CLA	2		1				0.67

*Below each seizure score, the number of rats reaching the score are shown.
Bicuculline (60ng) was infused over a two minute period into PC. 2CLA was microinjected fifteen minutes before the convulsant infusion. Seizure scoring was as described previously (Piredda & Gale, Nature 317:623-5). Supported by NIH grant NS-23227.

453.11 **BENZTROPINE COTREATMENT PREVENTS THE DEVELOPMENT OF HALOPERIDOL-INDUCED BEHAVIORAL HYPERSENSITIVITY BUT NOT THE DA RECEPTOR PROLIFERATION.** H.L. Klawans,P.M. Carvey, C.G. Goetz, C.M. Tanner and A. Hitri. Dept. Neurological Sciences, Rush-Presbyterian-St.Lukes Medical Center, Chicago,IL,60612.

Withdrawal from chronic treatment with neuroleptic agents such as haloperidol (HAL) results in an increased stereotypic behavioral (SB) response to dopamine (DA) agonists termed behavioral hypersensitivity (BH). BH has been utilized as an animal model of neuroleptic-induced tardive dyskinesia (TD). The relationship between BH and TD has been criticized recently because post-mortem studies of neuroleptic treated patients not exhibiting TD during their life still revealed DA receptor proliferation. We demonstrate here that a similar profile (receptor proliferation without BH) can be observed in the rat.

Compared to saline treated controls, rats chronically treated with HAL (0.75 mg/kg/28 days) exhibited a 71% increase in their SB response to apomorphine (0.75 mg/kg) 96 hours following treatment. The number of striatal spiroperidol binding sites (D-2) was increased by 32% and striatal DA and HVA content was reduced. Animals cotreated with benztropine (1.8 mg/kg: BENZ) and HAL also exhibited a 35% increase in spiroperidol binding sites and a reduced striatal DA and HVA content but were behaviorally normosensitive to apomorphine challenge.

These results demonstrate that DA receptor proliferation can occur without BH and, in this regard, BH is analagous to TD. These results further suggest that mechanisms other than just DA receptor proliferation are involved in BH, and perhaps by extension, TD. Since trihexyphenidyl and scopolamine cotreatments have also prevented the development of HAL-induced BH, muscarinic receptor proliferation may represent a compensating factor. QNB and 2-deoxyglucose studies will be presented to evaluate this possibility.

453.12 ACTH RECEPTOR DEFECT IN ADRENOLEUKODYSTROPHY (ALD). E.M. Smith*, G.E. Richards*, N.G. Greger*, A. Jazayeri*, P.G. Brosnan**, B.S. Keenan*, W.J. Meyer, III*, (SPON: G. Deutsch). Univ. of Texas Med. Branch, Depts. of Psychiatry and Pediatrics, Galveston, TX.

ACTH insensitivity is a cardinal feature of ALD, but the mechanism is unknown. We studied three boys in their early teens with ALD documented by increased plasma very long chain fatty acids, i.e., C26 = 1.57 ± .04 µg/ml, (control = 0.33 ± 0.18 µg/ml); C26/C22 = 0.05 ± 0.015 (control = 0.01 ± 0.01). All had a history of seizures. One had a normal neurlogic exam; his EEG showed non-specific slowing with normal auditory evoked responses: and his Magnetic Resonance Imaging showed areas of increased signal intensity in the cerebral peduncles and the internal capsule. The other two had severe progressive neurological deterioration. Basal ACTH concentration was elevated and plasma cortisol did not increase significantly with exogenous ACTH. Basal and stimulated renin and aldosterone were normal. The children's mononuclear leukocytes had no detectable high affinity ACTH binding sites in a radioligand binding study. In contrast, normal mononuclear leukocytes possess high (Kd = .04 nM) and low (Kd = 3.4 nM) affinity receptors for ACTH that appear identical to the prototype adrenal receptors (Bost and Blalock, Mol Cel Endocrinol 44:1, 1985). Mononuclear leukocytes from both ALD patients and controls had normal beta adrenergic receptors. In normal cells the high affinity ACTH receptors, but not beta adrenergic receptors, can be blocked by incubating with hexacosanoic acid (C26). These studies suggest that the adrenal failure associated with ALD is secondary to an aquired ACTH receptor defect caused by the excess long chain fatty acids. We speculate that ALD is the first example of an non-antibody related acquired polypeptide receptor defect.

BRAIN METABOLISM IV

454.1 A ^{31}P-NMR STUDY OF BRAIN CREATINE PHOSPHATE DEPLETION IN MICE FED AN ANALOGUE OF CREATINE. D. Holtzman, E. McFarland*, T. Moerland*, J. Koutcher*, M. Kushmerick*, and L.J. Neuringer*. Francis Bitter National Magnet Laboratory, M.I.T., Cambridge, MA 02139.

It is generally accepted from studies of muscle energetics that phosphocreatine (PCr) is an energy store available to buffer changes in ATP concentration (Meyer et al., Am. J. Physiol. 250: C264-C274, 1986). In ischemic or rapidly contracting muscle, PCr concentration falls nearly to zero before ATP decreases. In the hypoxic or seizing brain, PCr concentration decreases about 50% and then is stable without any change in ATP (Prichard et al., PNAS 80: 2748-2751, 1983). If the brain then becomes ischemic, PCr and ATP concentrations fall together. One proposed explanation for this tissue difference is that the brain's cellular or subcellular heterogeneity provides compartments with different ATP-PCr exchange kinetics, thresholds, or controls (Thulborn et al., J. Cerebral Blood Flow Metab. 2: 299-306, 1982). To study the functional and metabolic roles of PCr in brain, albino mice were fed an analogue of creatine, β-guanidinopropionic acid (GPA). In muscle, this analogue depletes PCr by competitive inhibition of creatine transport. It is phosphorylated but only slowly hydrolyzed by creatine kinase.

Mice fed GPA were normal in appearance and behavior. In the first two weeks, the phosphorylated analogue (GPAP) concentrations increased and PCr decreased (relative to ATP) at similar rates in brain and skeletal muscle as measured by in-vivo ^{31}P-NMR spectroscopy. In skeletal muscle, GPAP almost completely replaced PCr with the GPAP/PCr ratio, reaching values of 10 after six weeks of feedings. In contrast, the ratio of GPAP/PCr in brain was stable at 0.6-1.0 in animals fed the analogue for periods of two weeks to ten months. pH was not altered in either tissue. As previously noted in skeletal muscle, saturation transfer studies of brain showed GPA phosphorylation to be much slower than that of creatine. When GPA feedings were stopped, GPAP decreased in muscle much faster than in brain.

These results suggest that brain, unlike muscle, may have several compartments of PCr definable by GPA-induced depletion. The GPA-accessible compartment may represent 30-50% of brain PCr, a quantity similar to that which is readily depleted during hypoxia and seizures and to the difference between the PCr concentration in the immature and adult brain. Supported by a NRSA to DH (NS 08039) and grants to MK (NIH, AM 32281 and Muscular Dystrophy Foundation) and to LJN (NIH, RR 00995).

454.2 AUTORADIOGRAPHIC EVIDENCE FOR AXONAL TRANSPORT OF DEOXYGLUCOSE Robert F. Ackermann and Michael E. Phelps
Department of Radiological Sciences, Division of Nuclear Medicine and Biophysics; and Department of Neurology; UCLA School of Medicine, Los Angeles, CA 90024

It is generally presumed that the energy requirements of any given brain structure are normally met almost exclusively by glucose transported locally from capillaries that course through the brain at 50-100 micron intervals. However, we have obtained evidence suggesting that either glucose or glucose-6-phosphate can be selectively transported from neuronal cell bodies to their axonal terminal regions. In the first experiment, 14C-2-deoxyglucose (2DG) (1 uCi in 0.2 ul) was injected directly into the hylar region of the ventral hippocampus of albino rats; the animals were decapitated 5, 24, or 48 hrs later, and their brains processed for contact autoradiography. Near the 2DG injection site, label was concentrated in the stratum granulosum, the stratum pyramidale, and in some cases subiculum. Far from the injection site, label was concentrated in the CA3-CA4 region of the rostral hippocampus, in fascicles of the fimbria-fornix, and in the lateral septal nucleus; all these structures are known to receive axons from the neuronal cell bodies labeled by the injection. In animals injected unilaterally, labeling was confined to the ipsilateral side, the contralateral hemisphere serving as a control for uptake of 2DG that diffused from tissue to blood and then recirculated to the brain. In some animals, kainic acid (1 nmol), a neuroexcitant known to increase cerebral glucose metabolism, was added to the injectate; kainate increased the intensity of labeling locally, along efferent tracts, and in terminal regions. In other groups of rats 2DG was injected unilaterally in the neocortex, caudate nucleus, or the eye. In all cases, label was subsequently detected in the appropriate terminal fields, respectively: corpus callosum and contralateral neocortex; striato-nigral bundle and substantia nigra (pars reticulata); contralateral optic tract, lateral geniculate body, and superior colliculus. Chemical identification of the molecules carrying label to terminals is in progress. However, these preliminary results present the possibility that axonal elements are supplied glucose not only exogenously from nearby capillaries, but also endogenously from their own cell bodies.

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454.3 RATE OF CEREBRAL GLUCOSE METABOLISM IN THE BRAINSTEM OF THE SQUIRREL MONKEY. K. R. Brizzee and W. P. Dunlap*. Dept. Neurobiology, Delta Regional Primate Research Center, Covington, LA 70433 and Dept. of Psychology, Tulane University, New Orleans, LA 70112.

The squirrel monkey (Saimiri sciureus) is being used increasingly as a model for the study of metabolic processes in the human brain. The [^{14}C]2-deoxyglucose method has proved to be a powerful tool in mapping structures which may be specifically activated by physiological or pharmacological stimuli. The present study is to determine the baseline rates of glucose utilization in the squirrel monkey preliminary to its application in subsequent experimental studies. The femoral artery and vein were cannulated under general halothane-nitrous oxide anesthesia in five adult female squirrel monkeys. The local femoral area of the incision was maintained under lidocaine analgesia for the remainder of the experiment. Five hours after regaining consciousness from the general anesthesia the animals were restrained on a modified restraint device (Withrow and Devine, Lab. Anim. Sci. 22:419, 1972) and [^{14}C]2-deoxyglucose at a dose of 20 μCi/100 grams body weight was injected via the femoral venous cannula. Thirteen timed arterial blood samples (250 μL) were drawn in a period of 45 minutes, and plasma levels of [^{14}C]2-deoxyglucose and glucose were determined. The animals were then sacrificed with an overdose of nembutal. The brains were quickly dissected out and frozen in liquid nitrogen for 12 seconds and then placed in a dry ice chamber. Transverse cryostat sections were cut at 20 μm throughout the medulla oblongata, pons and midbrain. Autoradiographs were prepared on SB-5 X-ray film with a 10-day exposure. Densitometry was performed on the autoradiographs of sections from brainstem structures from level P8.0 to level A3.0 of a stereotaxic atlas of the brainstem (Emmers and Akert, 1963). The data were compiled using the operational equation developed by Sokoloff et al (J. Neurochem. 29:897, 1977). The mean glucose utilization rates (μmol/100gm/min.) for several of the major brainstem structures were as follows: Medial vestibular nucleus 66.0; Lateral vestibular nucleus 55.1; Inferior vestibular nucleus 49.8; Superior vestibular nucleus 38.0; Oculomotor nucleus 36.6; Trochlear nucleus 29.3; Substantia nigra (compacta) 28.5; Dorsal raphe nucleus 27.8; Abducens nucleus 26.8; Nucleus gracilis 25.4; Substantia nigra (diffusa) 25.0; Nucleus cuneatus 24.3; Nucleus trigeminospinal tract 24.0; Area postrema 22.2; Locus coeruleus 20.6; Hypoglossal nucleus 18.6; Lateral parabrachial nucleus 16.1; and Corpus restiformis 6.3.

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454.4 EFFECTS OF CHRONIC NICOTINE ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT. R. J. Fanelli and E. D. London. Neuropharm. Lab., NIDA Addiction Research Center, Baltimore, MD 21224.

Previous work from our laboratory has shown that nicotine (N) acutely stimulates local cerebral glucose utilization (LCGU) selectively in brain regions with high densities of specific nicotinic cholinergic binding sites. Because N is self-administered chronically, it is important to know its effects on brain function following repeated administration. We used the 2-deoxy-D-[1-^{14}C]glucose (DG) technique of Sokoloff et al., (J. Neurochem. 28:897, 1977) to measure LCGU, an index of local brain function, in rats treated chronically with N. In a completely random 2 X 2 factorial design, rats received s.c. injections of N tartrate (1.0 mg/kg as the base) or 0.9% NaCl twice daily (0900 and 1700 h) for 10 days. They were observed for the presence of selected behaviors (e.g., tremor, ataxia, hyperactivity) at 5, 15 and 30 min after treatment. On the morning of day 11, rats were given the same treatment as on previous days, and then were prepared with indwelling femoral venous and arterial catheters. Following a 3 h recovery period, they received either 0.3 mg/kg N or NaCl s.c., 2 min before DG (100 μCi/kg, i.v.). Blood pressure, pulse rate, blood gases, and body temperature were measured periodically during the DG experiments. Animals were killed 45 min after the DG injection, and LCGU was determined autoradiographically as described by Sokoloff et al. (1977).

The duration of some of the N-induced behaviors decreased over the 10 days of drug treatment, but behavioral tolerance was not clearly indicated. The frequency and severity of ataxia actually increased with repeated N exposure. Animals given N for the first time on the DG experiment day showed LCGU stimulation similar to that which we observed previously with acute N treatments. Increases occurred in brain regions such as the anteroventral thalamus, fasciculus retroflexus, substantia nigra pars compacta, and interpeduncular nucleus. Following chronic N, some brain regions (anteroventral thalamus, substantial nigra pars compacta) showed evidence of tolerance in the LCGU response to N challenge on the day of the DG experiment. Several brain regions that showed no increase after acute treatment (frontal cortex, subiculum) had increased LCGU following chronic N, perhaps indicative of receptor upregulation in these regions. In the group treated chronically with N but given NaCl on the day of the DG experiment, several brain regions (interpeduncular nucleus, superior colliculus) had LCGU values lower than the group given NaCl alone, which may be related to withdrawal. These findings provide important information regarding the local effects of chronic N, and begin to provide an anatomical basis for behavioral tolerance despite nicotinic receptor upregulation in the brain.

454.5 REGIONAL CEREBRAL GLUCOSE UTILIZATION DURING CHRONIC HYPO- AND HYPERGLYCEMIA IN THE RAT. D.A. Pelligrino* and A.S. Sharp* (SPON: W.E. Hoffman), Dept. of Anesthesiology, Michael Reese Hosp. and Med. Center, Chicago, IL 60616.

Reports on the effects of chronic blood glucose changes on regional cerebral glucose utilization (rCMRglu) are virtually absent from the literature. In the present study, we investigated in rats the influence on rCMRglu of 2 weeks of moderate hypoglycemia (plasma glucose = 2.8 ± 0.1 u mol.g^{-1}) and 3-4 weeks of pronounced hyperglycemia (plasma glucose = 29 ± 1.6 u mol.g^{-1}). Control rat plasma glucose was 10.1 ± 3.4 umol.g^{-1}. The rats were rendered hypoglycemic via the subcutaneous implantation of an osmotic pump (Alzet) that delivered 2.5-2.7 U/day of insulin. Chronic hyperglycemia was produced by suspension of insulin treatment in genetically diabetic (BB/W) rats. rCMRglu was measured employing a sequential double-label (^{3}H and ^{14}C) 2-deoxyglucose (DG) technique (Cunningham, V.J. Brain Res. 221:319, 1981). The regions evaluated were: frontal cortex (FC), sensorimotor cortex (SM), caudate-putamen (CP), hypothalamus (HT), amygdala (A), hippocampus (HC), thalamus (T) and cerebellum (CB). For evaluation the rats were surgically prepared with arterial and venous catheters under 0.7% halothane, 70% N_2O/bal. O_2 while paralyzed and artificially ventilated. The halothane was switched off > 1 h prior to study. The DG was injected iv, with 2 min between injections. The brain was fixed 5 min after the initial injection via focused microwave irradiation (10 kw, 0.7 sec). The regions were analyzed for free DG, DG-phosphate and glucose. The results, expressed as percent of control, are presented in the table below:

	rCMRglu (% control)							
	FC	SM	CP	HT	A	HC	T	CB
Hypogly.	56	62	70	91	66	92	100	91
Hypergly.	66	71	88	110	87	74	80	67

The hyperglycemic results are generally consistent with the hypothesis of a reduction in glycolytic rate in the presence of increased cerebral ketone body utilization. Further analysis of the hypoglycemic data indicated an inverse relationship between the magnitude of the rCMRglu change and the normoglycemic rCMRglu among regions. This agrees with previous results in acutely hypoglycemic rats (plasma glucose = 2 umol.g^{-1}) (Bryan, R.M. J. Neurochem. 46:1904, 1986) where it was also suggested that rCMRglu in hypoglycemia is eventually limited by blood to brain glucose transport. However, a comparison of rCMRglu changes in chronically vs acutely hypoglycemic rats indicates a greater reduction in the former (at a higher plasma glucose) and calls into question the significance of the recently reported increase in blood to brain glucose transport capacity in chronically hypoglycemic rats (McCall A.L. Am J. Physiol. 251:E442, 1986). Supported by American Diabetes Association.

454.6 REGIONAL CEREBRAL BLOOD FLOW DURING CHRONIC HYPOGLYCEMIA IN RATS. R.M. Bryan and D.A. Pelligrino. Departments of Surgery (Neurosurgery) and Physiology, The M.S. Hershey Medical Center of The Pennsylvania State University, Hershey, PA 17033; and Department of Anesthesiology, Michael Reese Hospital and Medical Center, Chicago, IL 60616.

Regional cerebral blood flow (rCBF) was measured during chronic hypoglycemia in rats. The results were compared to those from a previous study where rCBF was measured during acute hypoglycemia. Alzet mini-osmotic pumps were implanted in adult male Sprague-Dawley rats. The pumps delivered approximately 3 units of insulin per day days and maintained plasma glucose concentrations at 2.8 ± .2 umol/ml (50 ± 4 mg/dl) for 5-7 days. Plasma glucose was measured daily to ensure that the pumps were working consistently. Control rats either had sham-pumps implanted or had no pumps implanted. On the day of the study, the rats were anesthetized with nitrous oxide/halothane and catheters were placed in one femoral artery and one femoral vein. Plaster casts were fitted around the hip-region to restrain the rats and protect the catheters. Body temperature was monitored and maintained. Anesthesia was discontinued and each rat was allowed at least 2 hours to recover. Regional CBF was measured using ^{14}C-iodoantipyrine. Following surgery, plasma glucose rose to 4.5 ± .4 umol/ml (81 ± 7 mg/dl) in the chronically hypoglycemic rats. Insulin (1.5 units iv) was given to each hypoglycemic rat and rCBF was measured when plasma glucose fell to 2.2 umol/ml (40 mg/dl); approximately 10 min after the insulin injection. Plasma glucose in the normoglycemic control rats was 9.8 umol/ml (176 mg/dl). Regional CBF in all cortical regions studied (visual, auditory, sensory, olfactory, and motor) was significantly increased by 30-45% when compared to the rates in the normoglycemic controls. The only other brain regions to show elevated rCBF was the thalamus (29%) and pyramidal tract (20%). The effects of chronic hypoglycemia was, therefore, limited primarily to the cortical regions. On the other hand, results from previous studies show that the same level of hypoglycemia when produced acutely was accompanied by a more general increase in rCBF. Furthermore, the increases in rCBF in the cortical regions were greater during acute hypoglycemia than during chronic hypoglycemia. Since chronic hypoglycemia affects rCBF differently than acute hypoglycemia, we conclude that adjustments in the regulation of rCBF occurred in the chronically hypoglycemic rats. [Supported by a grant from the American heart Association (RMB), PHS grant NS 19341 from the National Institute of Neurological, Communicative Diseases, and Stroke (RMB), and a grant from the American Diabetes Association (DAP).]

454.7 EFFECTS OF COMPRESSION CONCUSSION ON BRAIN MITOCHONDRIAL BIOENERGETICS IN THE RAT

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Closed head injury (CHI) is a major cause of morbidity and mortality in the adult and pediatric population. Development of the most appropriate methods of treatment depends upon a complete understanding of the underlying pathophysiology of the injury. We attempted to clarify the effects of CHI and temporal sequence on cerebral bioenergetics in the rat.

Male Sprague-Dawley rats were anesthetized, a tracheal cannula placed and body temperature controlled at 37° using a heating pad and temperature probe. EEG was recorded via skull screws and systemic blood pressure monitored through an intra-arterial catheter. Compression-induced concussion was administered with a General Value Corporation Picospritzer II using nitrogen pulses at controlled pressures and time durations. At varying time periods (0, 1,2 and 3 hours) post-injury the animals were sacrificed and brain mitochondria were isolated according to a modification of the method described by Fiskum, 1984. Rates of oxidative-phosphorylation for pyruvate + malate and succinate + rotenone were determined polarographically using a Clark oxygen electrode and compared with tandem control rat brain mitochondria.

Our results revealed a 60-70% diminution of respiratory control indices for both mitochondrial substrates only in the 3 hour post-concussion rat, indicating a significant degree of uncoupling occurred. These preliminary results show that it may be possible to initiate a specific treatment regimen prior to the onset of mitocondrial dysfunction

454.8 ANOXIA AND CNS WHITE MATTER: IN VITRO STUDIES USING THE RAT OPTIC NERVE. P. Davis* and B.R. Ransom. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The cellular consequences of anoxia have been best studied, to date, in grey matter. We analyzed the effects of anoxia on mammalian CNS white matter, in adults and during development, using the isolated rat optic nerve (RON). The compound action potential (CAP) was used as a monitor of RON excitaiblity to quantify the functional recovery of the RON following anoxia or other treatments. RONs of different ages (1 to 90 days) were dissected free, placed in a recording chamber and bathed at 37°C with a bicarbonate buffered saline solution containing 5 mM $[K^+]_o$.

In the adult RON, CAP amplitude fell to 0 within 2-3 minutes of anoxia onset. CAP recovery was judged 60 minutes following the period of anoxia; in most instances maximum recovery was achieved within 30 minutes. CAP recovery was a linear function of the anoxic interval. Periods of anoxia less than 15 minutes were followed by 80-90% recovery, 60 minutes of anoxia by an average of 34% recovery, and only after 75 minutes of anoxia was recovery absent. RONs from animals less than 7 days of age were resistant to anoxia; CAP amplitude did not change with anoxia (up to 90 minutes). Sensitivity to anoxia, measured as incomplete recovery from a standard 60 minute anoxic insult, developed gradually from postnatal day 10 to 20 and remained constant thereafter.

Using a standard 60 minute anoxic insult, adult RONs were examined for factors which could influence degree of functional recovery. When the glucose concentration was increased from 10 mM, which is standard for many physiological solutions, to 20 mM, CAP amplitude fell more slowly, and maximum CAP recovery increased from a mean of 31 ± 15% to 56 ± 14% ($P > .025$). In some experiments $[K^+]_o$ and extracellular pH (pH_o) were monitored in the RON using ion-sensitive microelectrodes. These studies revealed that the higher glucose concentration was associated with a delayed and reduced rise in $[K^+]_o$ as well as an enhanced acid shift. Enhanced anaerobic energy metabolism, with greater lactic acid production, may explain these changes. The delayed rise in $[K^+]_o$ may account for the delayed loss of excitability seen with 20 mM glucose. Reducing $[Ca^{++}]_o$ from 2 to 0 mM in the bathing solution increased recovery from 31 ± 15% to 86 ± 4 ($P < .001$). Exposure for 60 minutes to 10 mM glutamate, an endogenous excitotoxin, had no effect on CAP amplitude during or after the exposure.

These studies have begun to define the characteristics of anoxic pathophysiology in mammalian white matter. This tissue is relatively resistant to anoxia, compared to grey matter, may be partially protected from anoxia by increases in extracellular glucose concentration, and appears critically dependent upon $[Ca^{++}]_o$ with regard to the degree of irreversible anoxic injury.

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454.9 BRAIN EDEMA AND FUNCTIONAL RECOVERY FOLLOWING FOCAL ISCHEMIA IN THE RAT. Teiji Tominaga*, Mitsue Tominaga*, Chika Katagi* and S. Tsuyoshi Ohnishi (SPON: S. Shuman). Membrane Research Institute, University City Science Center, Phila., PA 19104.

In order to study the relationship between ischemic brain edema and functional recovery, ionic movement, water content and EEG activity were investigated using a rat focal ischemia model. Chlorpromazine (CPZ) was reported to have a beneficial effect on tissue ischemia. Therefore, we studied the effect of this compound. Using Sprague-Dawley rats, the focal ischemic lesion in the territory of the right middle cerebral artery (MCA) was produced according to Chen et al. (Stroke 17:738, 1986). The sham control group served as a pre-ischemic condition. On the 1st, 3rd or 7th day after operation, animals were sacrificed and water content was measured by the wet-dry weight method. Then, sodium, potassium and calcium contents were determined by atomic absorption (Young, W., et al., CNS Trauma 3:215, 1986). EEG was recorded before and one hour after MCA occlusion under artificial ventilation. On the 3rd and 7th day, EEG was recorded from the same animal in a similar manner and analyzed by Fourier transform. Water content in non-treated and CPZ-treated groups is shown in the Table. Significant increases were obtained in the ipsilateral hemisphere concurrent with accumulation of sodium. Calcium content was increased (Right/Left ratio was 1.40 in Day 3). CPZ suppressed both the water increase and electrolyte movement. In EEG analysis, the power ratio between pre-ischemia and post-ischemia was improved in the treated group. A certain type of potassium blocker had a protective effect against brain edema.

Table: Water content (weight %) in non-treated and CPZ-treated (20mg/kg,i.p.) groups

	Non-treated group		CPZ-treated group	
	Right	Left	Right	Left
Sham	79.32±0.09	79.40±0.13	-	-
Day1	81.45±0.49*	79.31±0.16	81.05±0.58	79.10±0.18
Day3	81.41±0.14*	79.46±0.23	80.26±0.11*	79.20±0.39
Day7	80.43±0.45*	80.02±0.34*	79.37±0.18*	78.56±0.07*

*Significantly different from sham or non-treated group ($P<0.05$).

454.10 INVOLVEMENT OF PLATELET-ACTIVATING FACTOR IN CENTRAL NERVOUS SYSTEM DISORDERS. F. Clostre, C. Millerin, C. Betin and P. Braquet I.H.B. Research Labs, 17 avenue Descartes F-92350 Le Plessis-Robinson (France) (SPON: J.R. Boston)

A potential role for Platelet-Activating Factor (PAF) in the central nervous system was suggested by the discovery that triazolobenzodiazepines (e.g. alprazolam, triazolam, WEB 2086) and to a lower extent some benzodiazepines, antagonize PAF (rev. in Braquet et al. Pharmacol. Review, in press). PAF or PAF-like phospholipids may be involved in cell-to-cell interactions in nature brain, one observation supporting this suggestion being the lack of enzymes in Zellweger syndrom. Alprazolam is used as an antidepressant agent particularly in the treatment of panic disorders.

Ginkgo biloba extract (GBE 761) is used clinically for prevention of stroke and psycho-behavioural problems of senescence. Potent and specific PAF-antagonists have been found in Ginkgo biloba (Braquet, Lancet, 1985 ; i : 1501) ; termed Ginkgolides (G), the range of activity of these antagonists on [^{3}H] PAF binding assay is the following : BN 52021 (GB) > BN 52020 (GA) >> BN 52022 (GC) > BN 52024 (GJ). We therefore undertook the investigation of the role of Ginkgolides in several behavioural tests.

In mice, the administration of Ginkgolides does not modify the behavioural pattern (open field, tetrabenazine test, electroconvulsive shock) and does not induce myorelaxation. In contrast, in the despair test in the mouse, BN 52021 (1 → 20 mg/kg p.o.), even at the lower dose significantly reduced the immobility time of the animals [control : 200.4 ± 17.1 sec ; 1 mg/kg : 157.8 ± 15.1 sec ; 10 mg/kg : 117.5 ± 9.4 sec ; 20 mg/kg : 84.2 ± 8.9 sec]. A similar result is obtained with alprazolam. The range of activity is BN 52021 > Alprazolam ≈ BN 52020 > BN 52022 > BN 52024 which corresponds to the range of activity versus PAF-receptor.

Furthermore, the protection exerted by BN 52021 is abolished by a β-adrenergic blocker : propranolol, a β_1-adrenergic blocker : atenolol, a β_2-adrenergic blocker : butoxamine, a dopamine antagonist : pimozide and $5HT_2$-antagonist : ketanserine. The effects of BN 52021 in the forced swimming test in mice is enhanced by pretreatment with a phosphodiesterase inhibitor, IBMX. Our data demonstrate that the desinhibitory effects of the PAF-antagonists (both Ginkgolides and alprazolam) on the forced swimming-induced behavioural despair of mice may be explained by the relathionships between PAF, β and $5HT_2$ receptors and cAMP cascade system.

454.11 **BENIFICIAL ROLE OF GINKGOLIDES (PAF-ACETHER ANTAGONISTS) AND CICLOSPORIN IN CEREBRAL ISCHEMIA.** B. Spinnewyn, N. Blavet, M-T. Domingo, P.E. Chabrier, N. G. Bazan*, P. Braquet. IHB Res. Labs. 17 Avenue Descartes F-92350 Le Plessis Robinson, France. *Louisiana State University Eye Center, New Orleans, LA 70112.

Platelet-activating factor (PAF) is a potent mediator of anaphylaxis and shock. In addition, evidence for PAF participation in the gastric, intestinal and heart post ischemic phase has been recently found. Ginkgo biloba extract improves cerebral metabolism and protects brain against hypoxic damage in various models of cerebral ischemia. Potent and specific antagonists of PAF have been found in Ginkgo biloba; termed Ginkgolides: BN 52021, BN 52020, BN 52022 and BN 52024. We therefore undertook the investigation of the role of Ginkgolides in cerebral ischemia obtained by bilateral ligature of the common carotids for 10 min and 6 hours of recirculation in male Mongolian adult gerbils. Given preventively (one week treatment 10 mg/kg/day orally) BN 52021 and related ginkgolides dose-dependently antagonize morbidity assessed by the stroke index. Similarly the mitochondrial respiration evaluated by the respiratory control ratio [RCR; State 3/State 4 of the respiratory chain] is significantly improved. R.C.R. sham operated: 6.08±0.11; ligatured control: 4.50±0.23 BN 52020: 5.01±0.15; BN 52021: 5.67±0.18; BN 52022: 4.67±0.09; BN 52024 4.21±0.53. In both determination, the range of activity: BN 52021>BN 52020>>BN 52022>BN 52024 shows that ginkgolide effects in cerebral ischemia are correlated with their PAF-acether antagonistic properties [RCR=-0.98 log ($1/IC_{50}$ (^{3}H)PAF binding assay) +10.25; r=0.99]. Given curatively, one hour after declamping, BN 52021 (10 mg/kg, i.p.) and ciclosporin (20 mg/kg, p.o.) to a lesser extent induce a significant protection. [R.C.R. sham operated 6.09±0.10; ligatured control: 3.99±0.29; BN 52021 5.86±0.54; ciclosporin: 4.94±0.18]. These results give support to the presence of functionnal receptor of PAF-acether in gerbil brain. Saturation and competition experiments using [^{3}H]PAF have demonstrated reversible, saturable and specific binding with a K_D of 0.1 nM displaceable by PAF and BN 52021 but not by lyso-PAF. Morever, this protection is accompanied by a decrease in lipid peroxidation, a recovery of potassium content and a normalization of sodium, and water levels, demonstrating that ginkgolides antagonize cerebral edema.

As evidenced by a preliminary electron microscopy study, the protection observed with BN 52021 and ciclosporin (which does not possess any PAF antagonist properties) may occur throught an inhibition of cell infiltration and degranulation occurring in cerebral post ischemic phase and the appearance of eosinophil cationic protein in cerebrospinal fluid, PAF being one of the most potent chemotactic agents for eosinophils.

CELL SURFACE MACROMOLECULES II

455.1 A PC-12 CELL SURFACE ANTIGEN WHICH INVOLVES OR REGULATES TRANSMITTER RELEASE. Y. Kuroda, K. Kobayashi and Y. Ohguchi. Dept.of Neurochem.,Tokyo Metropol. Inst. for Neurosci., Fuchu-shi, Tokyo 183, Japan.

A library of monoclonal antibodies against cell surface antigens of rat pheochromocytoma PC-12 cells was obtained. Spleen cells of mice injected with intact PC-12 cells were fused with myeloma. Culture fluid of the resulting hybridoma was first screened by enzyme-linked immunosorbent assay(ELISA). ELISA-positive cells were cloned and the monoclonal antibodies were purified from the serum-free medium. More than 70% of the monoclonal antibodies recognized the cell surface antigens. The monoclonal antibody library was, then, applied to in vitro formed cholinergic synapses between cultured neurons to see their effect on synaptic transmission. Among the antibodies added, only one monoclonal antibody (PCH 32-20) blocked the synaptic transmission.

The monoclonal antibody was examined to see its effect on in vivo synapses. Rat superior cervical ganglia were disected out. The pre-ganglionic fiber was electrically stimulated and the evoked EPSP were observed by a microelectrode in post-ganglionic neurons. The monoclonal antibody solution of PCH 32-20 decreased the amplitude of EPSP and the mean quantal content in a dose dependent manner. This monoclonal antibody stained a broad band around 24K daltons in SDS-PAGE western blot of PC-12 cell membrane preparation. These data suggest that the monoclonal antibody (PCH 32-20) binds to a antigen on presynaptic membrane of the ganglionic synapse and change its function, for transmitter release itself or for its regulation.

This work was supported by a Grant-in-Aid for Special Project Research on Mechanism of BioelectricaL Response (60115008) from the Japanese Ministry of Education, Science and Culture.

455.2 PURIFICATION AND CHARACTERIZATION OF AN ANTIGEN THAT IS SPATIALLY SEGREGATED IN THE PRIMARY OLFACTORY PROJECTION. J.E. Schwob and D.I. Gottlieb. Dept. Anat. and Neurobiol., Washington University School of Medicine, St. Louis, MO 63110.

The monoclonal antibody designated RB-8 heavily labels axons derived from the ventrolateral olfactory epithelium and their terminals in glomeruli of the ventrolateral olfactory bulb, but leaves the axons and terminals from the dorsomedial epithelium unstained or lightly stained (J. Neurosci. 6:3393). In the olfactory nerve RB-8 recognizes a 125 kDa, membrane-associated protein. RB-8 also reacts with a 125 kDa membrane protein elsewhere in the CNS and PNS. This antigen is nervous system specific and is found on some neurons but not others. We report here the purification of the 125 kDa RB-8 antigen and its further characterization as a cell surface glycoprotein.

To show that the RB-8 antigen is exposed on the cell surface, explants of the fetal olfactory epithelium in tissue culture were exposed to the RB-8 antibody while living, which results in labeling of the olfactory axons derived from the explants. The staining of living axons with RB-8 antibody is due to the surface localization of the antigen rather than disruption of the plasma membrane, since antibody to vimentin, a known internal antigen, does not label axons in parallel control cultures that are unfixed and living, but will label axons that have been fixed and permeabilized.

On the assumption that the antigen in brain is the same as the protein in the olfactory nerve, we used whole brain as the starting material for the purification of the RB-8 antigen. The antigen can be purified to homogeneity as follows: (1) the RB-8 antigen is extracted from brain membranes with deoxycholate; (2) The solubilized antigen is highly enriched by chromatography over an RB-8 antibody column; (3) The RB-8 antigen is separated from the other proteins in the column eluate by preparative one dimensional SDS-PAGE and then eluted from the 125 kDa band on the gel. The 125 kDa band in the column eluate is composed solely of immunoreactive protein as shown by immunoblots of two-dimensional gels.

The purified protein was shown to be a glycoprotein by its reactivity with lectin probes. In addition, the purified antigen was used to generate a polyclonal rabbit antiserum that recognizes only the 125 kDa protein on immunoblots. Immunohistochemical staining of the olfactory bulb with this antiserum exactly replicates the pattern of staining seen with the monoclonal RB-8 antibody. Thus, the 125 kDa protein from the whole brain shares strong immunological homology with the olfactory nerve protein. This additional characterization of the RB-8 antigen and the availability of a polyclonal antiserum may prove useful in assessing the functional role of the antigen in the primary olfactory projection. Supported by NIH, NS 08076 and NS 12867.

455.3 A SYNAPSE-SPECIFIC CARBOHYDRATE AT THE NEUROMUSCULAR JUNCTION IS ASSOCIATED WITH BOTH ACETYLCHOLINESTERASE AND A GLYCOLIPID. L.J. Scott* and J.R. Sanes. Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

We previously showed that the lectin DBA (Dolichos biflorus agglutinin) selectively stains neuromuscular junctions in rat skeletal muscle and thus defines a synapse-specific carbohydrate (Sanes and Cheney, Nature 300: 646, 1982). The existence of this carbohydrate and the relative simplicity of the neuromuscular junction encourage use of this system to study the involvement of saccharides in synapse formation and function. Using a panel of lectins, antibodies, and glycosidases of known specificities as histochemical probes, we found that the carbohydrate moiety concentrated at the neuromuscular junction resembles N-acetylgalactosamine (GalNAc) linked in the β anomeric form to the termini of oligosaccharides. We also found that a second lectin, VVA-B_4 (Vicia villosa B_4-isoagglutinin) stains synapses as selectively as, but more intensely than, DBA. Using VVA-B_4, we asked whether a similar carbohydrate is concentrated at skeletal neuromuscular junctions in other vertebrates. In fact, VVA-B_4 selectively stains neuromuscular junctions in most major vertebrate classes (12 species), a phylogenetic conservatism that suggests a synapse-related role for GalNAc β-terminal saccharides.

Further study of the synapse-specific carbohydrate requires identification of the molecules to which it is attached. We previously showed that DBA binds the asymmetric, collagen-tailed forms of acetylcholinesterase (which are highly concentrated at the rat neuromuscular junction) but not globular forms (which are more widely distributed; Scott and Sanes, Neurosci. Abstr. *10*: 546, 1984). VVA-B_4 also binds asymmetric but not globular acetylcholinesterase. We have now identified a second GalNAc-bearing glycoconjugate immunohistochemically, using a monoclonal antibody, anti-SSEA-3, which recognizes GalNAc-bearing lipids (Shevinsky et al., Cell 30: 697, 1982): anti-SSEA-3 stains neuromuscular junctions selectively. SSEA-3-immunoreactive material is glycolipid-like (chloroform/methanol extractable) and distinguishable from AChE, but staining is blocked by VVA-B_4. One of two antisera to globoside (a GalNAc terminal glycolipid) tested also stains synapses selectively. The association of a synapse-specific carbohydrate with at least two different synapse-specific molecules raises the possibility that the former plays a role in determining a property that the latter share, such as their concentration at the synapse. (Supported by NIH and MDA.)

455.4 IMMUNOCYTOCHEMICAL STUDIES OF CELL SURFACE MOLECULES OF MIGRATING NEURONS in vitro. W.A. Gregory, C.A. Mason and M.E. Hatten. Dept. of Pharmacology, New York, NY 10016.

In previous studies, we have used high resolution video microscopy to study the movement and cytology of cerebellar granule cells migrating along astroglial processes in a microculture system. Correlated electron and video microscopy of actively migrating cells has shown specialized junctions (termed interstitial densities) formed between glia and the somata of migrating neurons. These interstitial junctions are located at the neuronal soma-glial apposition and are characterized by filaments spanning between apposed membranes. These interstitial filaments appear to be contiguous with or transmembranous extensions of submembranous cytoskeletal filaments. Extracellular space (20nm) is dilated at these regions. Both microtubules and microfilaments are seen in the leading process, arrays projecting to the plasma membrane. Numerous coated vesicles are seen in the leading process of migrating cells suggesting possible endocytosis of cell surface ligands. Non-migrating granule cells form another type of junction, puncta adherentia, with astroglia.

The present experiments use immunocytochemical techniques at the EM level to define the distribution of cell surface ligands, molecules thought to mediate cell-cell and cell-substrate adhesion. Cultures are being stained with polyclonal antibodies to astrotactin, a molecule involved in granule neuron-glial cell contacts, NILE (L1, NgCAM), BSP-2 (N-CAM), laminin and a mammalian form of integrin, a cell surface receptor for both fibronectin and laminin. Primary antibodies are observed on lightly fixed cultures using FITC-labeled secondary antibody fluorescence or colloidal gold-labeled secondary antibody *preembedding* electron microscopic techniques.

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455.5 INITIAL INTERACTIONS BETWEEN CEREBELLAR GRANULE CELLS, ASTROCYTOMA LINES AND SUBSTRATA. David E. Weinstein*, Michael S. Shelanski and Mary E. Hatten (SPON: R. Liem). Dept. Pharmacology. N.Y.U. Med. Ctr., 550 First Avenue, New York, NY 10016.

Two families of adhesive ligands, cell-cell and cell-substratum, have been proposed to mediate cell contact interactions and cell spreading on culture surfaces, respectively. A question that has not been addressed is how cells choose between cell and/or substrate molecules. Here we have explored the behavior of astrocytoma cells given choices between granule cell neurons and substrates.

To investigate the initial cell-substratum interactions, purified granule cells were mixed with astroglia and cell surface membrane specializations were monitored with high resolution, time-lapse, video-enhanced differential interference microscopy.

When the human U-251 or mouse G26-24 astrocytoma cells are cultured on untreated glass coverslips, in essence depriving them of an adhesive ligand, they display extensive surface membrane activity characterized by the rapid protrusion and retraction of membrane blebs. These cells fail to settle to the glass surface, even after several hours. Treating the culture surface with either fibronectin or polylysine (100ug/ml) abolishes this blebbing activity and the cells rapidly spread. The addition of neurons to the astrocytoma cells causes a short period of blebbing which ends when cell-cell contacts are established with the granule cell; thereafter the astroglial cell attaches to the culture surface.

When astrocytoma cells are plated with neurons in the presence of the polyclonal antibody anti-astrotactin, which blocks neuron-glial interactions or the monoclonal antibody 1A3, which also blocks neuron-glial interactions, little blebbing is seen on the astrocytoma cell surface and the cells rapidly spread onto the culture surface. Similarly adding the monoclonal antibody D26, believed to recognize the fibronectin receptor, reduced astrocytoma cell blebbing is seen until neuron-glial contacts form. These experiments suggest that blocking either neuron-glial or glial-substrate adhesion promotes the alternative choice, thereby reducing the period of membrane blebbing.

These findings suggest that the exaggerated membrane activity we have observed in astrocytoma cells is involved in cell decision making events, and these choices are related to the availability of cell surface ligands such as astrotactin and integrin-like cell surface receptors for fibronectin and/or laminin.

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455.6 EXPRESSION OF JONES GANGLIOSIDES BY CEREBELLAR NEURONS AND GLIA *IN VITRO*. R. Mendez-Otero and M. Constantine-Paton Dept. of Biology, Yale University, New Haven, Ct. 06511.

A monoclonal antibody named JONES recognizes ganglioside antigens which are expressed in regions of the developing rat retina where both cells and processes are migrating (Constantine-Paton et al.,1986, Nature, 324:459). In the postnatal cerebellum, immunohistochemical studies revealed a differential distribution of JONES binding that is correlated whith the patterns of granule cell migration and axonal outgrowth (Mendez-Otero et al., Soc. Neurosc. Abstr. 12:317).

To examine the expression of this antigen on a cellular level we have used an *in vitro* system previously developed to study the role of astroglia in neuronal migration in the mouse (e.g. Trenkner and Sidman, 1977, J. Cell Biol.75: 915 and Hatten et al., 1984, J. Cell Biol., 98: 193). Cells were dissociated from P5-P6 cerebellum with trypsin and plated on either glass cover slips or tissue chamber/slides pretreated with polylysine. Cultures were fixed with 4% paraformaldehyde and double labeled with JONES and cell type specific antibodies. Astroglial cells were identified using either RAT-401 or antiserum against glial fibrillar acidic protein (GFAP) and neurons by tetanus toxin binding.

After 3 hr *in vitro* ≈60% of the cells in culture were stained with tetanus toxin and ≈20% were labeled with RAT-401. JONES antibody stained≈70% of the tetanus toxin positive and ≈10% of the RAT-401 positive cells. In 24-48 hr cultures most of the neurons were associated with astroglial cells in a pattern that resembled the association between Bergman-like cells and granule cells previously reported in these cultures. JONES stained intensely the neuronal cell bodies and their processes while the labeling in the glia was less intense and punctiform. The number of JONES labeled neurones remained constant in these cultures from 3-72 hr while the number of GFAP positive cells increased to ≈ 30% over the same period. After a week in culture there was a decrease in the number of JONES positive neurons and glia consistent with the decrease of JONES immunoreactivity and migration of these cells during development *in vivo*.

Our results show that JONES expression is not exclusively associated with a neuronal or glial phenotype but is expressed by a sub-population of neurons and glia. We hypothesize that the neurons and glia expressing JONES in these cultures might be the ones actively involved in the process of migration. Supported by Biomedical Research Support Grant RO7015 to Yale University, NIH HD 22498 to MCP and a CNPq/Brazilian Fellowship to RMO.

455.7 *IN VITRO* MODULATION OF JONES GANGLIOSIDES EXPRESSION ON RAT RETINAL FIBERS AND OPTIC NERVE GLIA. D.M. Bonafede and M. Constantine-Paton. Dept. of Biology, Yale University, New Haven, Ct 06511.

The Jones gangliosides (Constantine-Paton et al. 1986 Nature 324:459; Schlosshauer et al. Soc. Neurosc. Abstr. 12:85.5) are highly correlated both spatially and temporally with regions of cell and axon motility in the developing rat nervous system (Mendez-Otero et al. Soc Neurosc Abstr. 12:85.6) We are exploring factors which control the expression of these gangliosides.

In vivo the Jones antigen is present on cells and in the optic fiber layer of the developing retina. *In vitro* this antigen's expression on cell somata and neurites of retinal explants from E18-E21 embryos appeared to be modulated by the substrate on which they grew: explants grown on poly-l-lysine or laminin expressed Jones, while those grown on native collagen did not.

One of the natural substrates of retinal ganglion cell axons are optic nerve glia. The Jones antigen, apart from a brief appearence in the optic stalk at E13, is immunocytochemically and biochemically absent from the optic nerve. However, in dissociated P7-P14 optic nerve glia, we found that ~20% of the cells expressed the antigen within one day and for at least 4 days of culture on poly-l-lysine. Most of the Jones positive glia also expressed galactocerebroside. Then, to test the hypothesis that the presence of retinal fibers actually inhibits Jones expression by these glia, we co-cultured, on poly-l-lysine, freshly dissociated P14 optic nerve glia with 2-3 day old E19-E20 retinal explants. While the retinal fibers continued to express the Jones gangliosides, the optic nerve glia lying within 400μm of the explants' edges failed to express the same antigen. Such glia were not necessarily in contact with neurites. In contrast, 10-15% of the glia lying at distances >>400μm from the explants, cultured on the same coverslip, were still able to express these antigens.

Our results indicate that the expression of Jones gangliosides is continuously modulated by a complex of interactions between growing neurites and their substrates and, furthermore that only part of such interactions are contact mediated

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455.8 SPECIFIC INACTIVATION OF ACETYLCHOLINESTERASE BY CHROMOPHORE ASSISTED LASER ABLATION. Daniel G. Jay* (SPON: D.H. Hubel). Dept. of Neurobiology, Harvard Medical School, Boston MA 02115.

A general procedure for site directed inactivation of protein function has been developed. Enzymes were inactivated by binding of specific probes (e.g. antibodies or ligands) conjugated with a chromophore and exposing the samples to high intensity laser light at a wavelength of 635 nm; a wavelength near the chromophore's absorption maximum that is not significantly absorbed by cellular components. The only energy input into the system was due to the excitation and relaxation of the chromophore and this was effective in denaturing proteins that are vicinal to the dye without damaging other proteins in the sample. The dye Malachite green was used because of its optimal absorption maximum, fast relaxation time, high molar absorptivity and lack of photobleaching.

The method was demonstrated using biotinylated forms of alkaline phosphatase and B galactosidase with Malachite green conjugated streptavidin. Samples incubated with dye conjugated streptavidin and exposed to 635 nm laser light (50 mWatts for 5 minutes) showed greater than 80% inactivation of biotinylated enzyme activity without significantly affecting the activity of nonbiotinylated enzymes in the same sample.

Human erythrocyte acetylcholinesterase, a cell surface enzyme, was inactivated by incubating red blood cells with Malachite green labeled anti-acetylcholinesterase monoclonal antibody and subjecting the sample to 635 nm laser light. No damage to any other cellular components in the red blood cell was observed.

This procedure may be useful for the selective short term loss of single functions in living cells and may be generally applied to the molecular dissection of cellular processes.

455.9 DISTRIBUTION OF LAMININ AND INTEGRIN ALONG TRUNK NEURAL CREST PATHWAYS IN XENOPUS LAEVIS. Danuta Krotoski and Marianne Bronner-Fraser, Developmental Biology Center, University of California, Irvine, CA 92717

Interactions between neural crest cells and the extracellular matrix have been proposed to play an important role both in cell guidance and cell differentiation. In avians, neural crest cells have been shown to migrate actively on fibronectin (FN) and laminin (LM) substrates in vitro, and these molecules have been shown to colocalize along neural crest pathways in vivo. Recently, integrin, a cell surface receptor for FN and LM, has been identified. Integrin appears to be involved in neural crest migration, since antibodies directed against it perturb cranial neural neural crest migration when injected in vivo (Bronner-Fraser, Dev. Biol. 117:528 1986).

Here, we describe the distribution of laminin and integrin during neural crest migration in the Xenopus trunk in order to define the mechanisms which control neural crest migration in this species. Xenopus embryos are easily manipulable and, therefore, provide a convenient model for the analysis of vertebrate neural patterning. We have recently described neural crest pathways in Xenopus laevis using two new cell marking techniques and have also described the distribution of fibronectin along these pathways.

Laminin immunoreactivity was observed along neural crest pathways. In the transverse plane, LM staining was visible in the basal lamina around the neural tube, notochord, somites, dorsal aorta, epidermis, dorsal mesentery and lateral plate mesoderm. This distribution was similar to that of FN, but appeared more fibrillar and diffuse than LM staining. Integrin staining was also seen around the neural tube, notochord, somites, dorsal mesentery and on epidermal cells. Additionally, integrin immunoreactivity was detected on the surface of cells located along neural crest pathways: along the dorsal neural tube, between the neural tube and somites as well as in the dorsal fin. The intensity of integrin staining appeared to increase with development. In the longitudinal plane, neural crest cells exhibited a periodicity in their migration pattern, migrating primarily along the posterior portion of each somite. In contrast, integrin, LM and FN immunoreactivity appeared uniformly distributed along the notochord and somites prior to, during and after neural crest migration at the light microscopic level. Thus, though these molecules may play a permissive role, they did not exhibit the periodicity required to play a guiding role for neural crest migration. (Supported by NSF Grant BNS 86-07760).

455.10 LYM-11 ALLOANTIGEN EXPRESSION ON CNS ENDOTHELIAL CELLS AS A MARKER OF STRAIN ORIGIN J. Joseph*, R.L. Knobler, F.D. Lublin, D. Goldowitz, J. Marini and M.N. Hart*. Div. Neuroimmunology, Jefferson Med. Col., Phila., PA 19107, Dept. Path., Univ. Iowa Hosp., Iowa City, IA 52242

The Lym-11 antigen is believed to be the beta 2 microglobulin component of the class I (K and D) histocompatibility antigens. However, it is coded for by a gene on mouse chromosome 2 (Immunogenet. 11:441, 1980), in contrast to the other components of the class I antigens which are coded for by genes on chromosome 17. Allelic forms of Lym-11 exist, and can be used to differentiate cells of otherwise identical class I type. There is an antibody which recognizes the Lym-11 allele on lymphocytes of B10.S but not SJL mice. Previous studies have focused on the tissue distribution of Lym-11, by attempting to diminish its activity in a cytotoxicity assay after absorbing the antibody with different tissues. By this technique, brain did not reduce cytotoxicity, and it was concluded that Lym-11 was minimally expressed on brain. In the present analyses, we measured direct labeling of Lym-11 on the surface of cultured brain endothelial cells using a cell sorter. This was done before and after exposure to interferons, which are known to affect histocompatibility antigen expression. In addition we studied Lym-11 expression on frozen sections of brain. We demonstrate that B10.S endothelial cells in culture and in brain express this antigen but it is not present on cells from SJL mice. Furthermore, the levels of expression dramatically rise on B10.S cells after exposure to interferons. Since both B10.S and SJL mice share the $H\text{-}2^{s}$ class I haplotype at the K and D regions, this difference in Lym-11 expression allows distinction between cells originating from these two strains of mice. This strain distribution thus allows differentiation of B10.S from SJL endothelial cells, and will serve to identify these cells in allogeneic transplantation studies of brain tissue between these two different strains of mice.

456.1 CARDIAC-RELATED NEURONAL DISCHARGE IN THE BNST DURING SLEEP-WAKING STATES IN THE DRUG-FREE CAT. R.M. Harper, R.R. Terreberry, R.C. Frysinger, H. Ni* and M. Oguri*. Dept. of Anatomy & Brain Research Institute, UCLA, Los Angeles, CA 90024.

The bed nucleus of the stria terminalis (BNST) is a forebrain structure located near the anterior commissure that has descending projections similar to those of the central nucleus of the amygdala (ACE) (Holstege et al., Exp. Brain Res. 58:379, 1985). A proportion of ACE neurons discharge phasically with the respiratory and cardiac cycles (Pascoe and Kapp, Brain Res. Bull. 14:331, 1985; Frysinger et al., Soc. Neurosci. Abs. 10:615, 1984; Zhang et al., Exp. Neurol. 91:193, 1986). A proportion of BNST neurons also discharge phasically with the respiratory cycle, and this discharge is state related (Terreberry et al., Soc. Neurosci. Abs., 1987). This study determines whether BNST neurons share characteristics of cardiac-related discharge manifested by ACE neurons.

Bundles of fine-wire microelectrodes were stereotaxically placed bilaterally in the BNST, together with electrodes to record EOG, EEG, and nuchal and diaphragmatic EMG in five cats under pentobarbital anesthesia. After recovery from surgery, neuronal discharge, diaphragmatic and nuchal EMG, ECG, and EEG activity were recorded in the intact, unrestrained, drug-free cat during several sleep-waking cycles.

A total of 70 neurons were recorded, 31 of which were localized in the BNST. Cross-correlations revealed that 21 BNST neurons (67%) discharged phasically with the cardiac cycle in at least one sleep or waking state. Twenty-nine percent of the neurons recorded exhibited phasic discharge with the cardiac cycle across multiple sleep-waking states. Twenty-three percent of all correlations occurred during waking (AW) and 42% occurred during quiet sleep (QS), whereas 35% of all correlations were observed during rapid eye movement sleep (REM). None of these discharge correlations could be considered "strong," and only 24% of the correlations were "moderate." These "moderate" correlations were observed most frequently during QS (75%), compared with AW (25%) and REM (0%).

Neurons in the BNST discharge phasically with the cardiac cycle, and this discharge appears to be state dependent. Although the correlations between BNST neuronal discharge and the cardiac cycle are weaker than the correlations seen between BNST discharge and respiration, the BNST, together with the ACE, may affect cardiac patterning via projections to brainstem areas involved in cardiovascular modulation.

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456.2 RESPIRATORY-RELATED NEURONAL DISCHARGE IN THE BNST DURING SLEEP-WAKING STATES IN THE DRUG-FREE CAT. R.R. Terreberry, R.C. Frysinger, H. Ni*, M. Oguri* and R.M. Harper. Dept. of Anatomy & Brain Research Institute, UCLA, Los Angeles, CA 90024.

The bed nucleus of the stria terminalis (BNST) is a forebrain structure that projects heavily to the parabrachial pons, dorsal vagal complex, nucleus ambiguus, and the nucleus of the solitary tract. These projections overlap projections from the medial portion of the central nucleus of the amygdala (ACE). Retrograde studies demonstrate that a continuum of cells bridging the BNST and ACE are labeled following injections of retrograde tracers into brainstem sites (Holstege et al., Exp. Brain Res. 58:379, 1985), suggesting that the two areas may form a continuous structure. Neurons in the ACE discharge phasically with the respiratory cycle, and these relationships are state dependent (Pascoe and Kapp, Brain Res. Bull. 14:331, 1985; Zhang et al., Exp. Neurol. 91:193, 1986). The present study examines the relationship of BNST neuronal discharge to spontaneous changes in respiratory parameters across sleep-waking states.

Bundles of fine-wire microelectrodes were stereotaxically placed bilaterally in the BNST, together with electrodes to record EOG, EEG, and nuchal and diaphragmatic EMG in five cats under pentobarbital anesthesia. After recovery from surgery, neuronal discharge, diaphragmatic and nuchal EMG, ECG, and EEG activity were recorded in the intact, unrestrained, drug-free cat during several sleep-waking cycles.

A total of 70 neurons were recorded, of which 31 were localized in the BNST. Cross-correlations revealed that 20 BNST neurons (64%) discharged phasically with the respiratory cycle in at least one sleep-waking state. Thirty-two percent of the neurons recorded exhibited phasic discharge with respiration across multiple sleep-waking states, most frequently across quiet waking (AW) and quiet sleep (QS). Forty-three percent of all correlations occurred during AW and 41% occurred during QS, whereas only 17% of all correlations were observed during rapid eye movement (REM) sleep. Only a small percentage (19%) of these discharge correlations with the respiratory cycle could be considered "strong." These strong correlations were observed only during either AW or QS.

Neurons in the BNST discharge phasically with the respiratory cycle, and this discharge appears to be state dependent. The BNST appears to be similar to portions of the ACE both anatomically and functionally and, together with the ACE, may constitute a limbic arousal system that provides excitation to brainstem areas subserving respiratory patterning.

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456.3 RESPIRATORY AFFERENT PROCESSING IN THE NUCLEUS OF THE SOLITARY TRACT IN RAT. D.R. McCrimmon, A.C. Bonham and D.O. Nelson Departments of Physiology, and Anesthesia, Northwestern University, Chicago, IL. 60611.

Pulmonary afferents are known to project to the nucleus of the tractus solitarius (NTS). We have previously shown (Fed. Proc. 46:1420 1987) that injections of small volumes of excitatory amino acids into the NTS, medial to the tractus solitarius, produce site-specific alterations in respiratory motor output and arterial pressure which resemble the responses to activation of pulmonary and baroreceptor afferents. These studies have been extended to characterize the neuronal activity in the regions from which the respiratory responses were elicited. Experiments were performed in urethane-anesthetized rats in which the diaphragm electromyogram (EMG) or phrenic nerve activity were recorded. End-tidal CO_2, arterial pressure and heart rate were continuously monitored. In some experiments rats were paralyzed with gallamine and mechanically ventilated. Single or multibarrel pipettes were used for unit recording and pressure ejection of excitatory amino acids and dyes for marking recording locations. Injection of 1 to 6 nl of DL-homocysteine (DLH, 10 mM, pH = 7.4) into the medial aspect of the nucleus of the tractus solitarius 200 µm caudal to the obex produced a transient apnea averaging 12±4 sec in 15 animals. Recordings of single unit activity in this region, with the same electrode from which DLH was ejected, identified neurons which discharged action potentials in phase with diaphragm activity in spontaneously breathing rats. To determine whether these neurons received pulmonary stretch receptor input, lung inflation was manipulated in paralyzed, mechanically ventilated animals. In these studies, DLH injection still produced transient periods of phrenic nerve silence. In addition, neurons within the DLH-sensitive region: 1) discharged action potentials during periods of lung inflation, 2) discharged with no obvious phase relationship to phrenic nerve activity, and 3) remained silent when the ventilator was turned off for three consecutive phrenic nerve bursts. In other proximal sites in which respiratory unit activity was recorded, turning off the ventilator had no effect on the phase relationship of unit discharge to phrenic nerve activity and DLH injection did not silence phrenic nerve activity. These results suggest that neurons in the reflex pathway from pulmonary stretch receptors are concentrated within the region from which DLH injections elicit apnea. Future studies will attempt to identify whether rapidly adapting and/or slowly adapting stretch receptors may be providing synaptic input to the neurons within this region. (Supported by NIH grants RR-05370, HL29033 and NS07243).

456.4 ACTIVITY OF INSPIRATORY NEURONS IN THE DORSAL AND VENTRAL RESPIRATORY GROUPS DURING FICTIVE VOMITING. A.D. Miller, L.K. Tan and S.F. Lakos*. The Rockefeller University, New York, N.Y. 10021.

Vomiting is primarily produced by the coordinated action of the major respiratory muscles (1). We sought to determine whether inspiratory neurons in the dorsal and ventral respiratory groups (DRG, VRG) have the appropriate firing pattern to activate phrenic motoneurons during vomiting. Neuronal activity was recorded from decerebrate, paralyzed cats during fictive vomiting, that is during a characteristic pattern of co-activation of phrenic and abdominal nerves, elicited by emetic stimulants, that would be expected to produce vomiting if the the animals were unparalyzed (2). Only neurons that were antidromically activated from the (C4) spinal cord were studied.

We have tested 9 inspiratory neurons in the DRG and 7 in the VRG. Most (15/16) of these neurons do not have the appropriate response pattern to initiate phrenic discharge during vomiting since the neurons are either silent when the phrenic nerve is active during fictive vomiting or else fire only at the end of phrenic discharge. These neurons would also not be expected to initiate external (inspiratory) intercostal muscle activity during vomiting since these muscles are co-active with the diaphragm (1). Therefore, although the number of neurons studied is still small, it would appear from these data that other descending inputs must be important for the control of the diaphragm during vomiting.

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1. McCarthy LE, Borison HL. Respiratory mechanics of vomiting in decerebrate cats. Am. J. Physiol. 226: 738-743, 1974.
2. Miller AD, Tan LK, Suzuki I. Control of abdominal and expiratory intercostal muscle activity during vomiting: role of ventral respiratory group expiratory neurons. J. Neurophysiol. 57(6), 1987.

456.5 INSPIRATORY NEURONS THAT ARE ACTIVATED WHEN INSPIRATION IS INHIBITED BEHAVIORALLY. John Orem. School of Medicine, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Respiration can be automatic or controlled behaviorally. Behavioral control in the cat occurs, at least in part, through control of the brainstem respiratory neurons that constitute the automatic system. Thus when inspiration is inhibited in response to a conditioned stimulus, inspiratory neurons in the medulla are inactivated. The latency of this inactivation ranged from approximately 40 to 120 ms and averaged 65 ms (±23 s.d.) across a sample of 28 inspiratory neurons recorded in the dorsal and ventral medullary respiratory groups. These inactivated inspiratory cells had strong and consistent respiratory signals during spontaneous breathing (mean η^2 = 0.51 ± 0.19, s.d., see Orem, J. and T. Dick, J. Neurophysiol. 50:1098, 1983 for an explanation of the η^2 statistic). In contrast to these inspiratory cells, six inspiratory cells and one expiratory- inspiratory cell were activated (mean latency = 36 ms ± 23, s.d.) when inspiration was inhibited behaviorally. These cells located in both the dorsal and ventral respiratory groups were recorded, in 6 of 7 cases, in the vicinity of inspiratory neurons that were inhibited during the behavioral response. These activated cells had low η^2-valued respiratory activity-- averaging only 0.27 across cells with a range of 0.03 to 0.60. Thus they receive considerable amounts of nonrespiratory input. It is proposed that these cells act as the interface through which behavioral inhibition of inspiration occurs. Supported by NHLBI Grant 21257.

456.6 PHRENIC INSPIRATORY ACTIVITY MODULATED BY STIMULATION OF PHRENIC SENSORIMOTOR CORTEX. F.J. Thompson, P.W. Davenport, J.J. Warner. Dept. of Neuroscience and Dept. of Physiological Sciences, Dept. of Neurology College of Med. and Vet. Med., Univ. of Florida, Gainesville, Florida 32610

We recently reported that stimulation of phrenic afferents evoked activity in the sensori-motor cortex in cats and proposed that these inputs may play a role in respiratory kinesthesia (Davenport, Thompson, Reep, and Freed, 1985). The purpose of the present study was to determine if these cortical areas represent low threshold regions for the control of respiratory movements.

These experiments were done on vagotomized adult cats anesthetized with alpha chlorolose (70 mg/kg). The contralateral sensorimotor cortical projection of phrenic afferents was mapped by analysis of sensory evoked potentials elicited by stimulation of the fifth cervical branch of the phrenic nerve. The mapped cortical areas were then stimulated to determine the influence of stimulation of the cortical phrenic projection areas on the inspiratory activity recorded in the phrenic nerve. The stimulus triggers were cycle triggered from the onset of the inspiratory activity. The influence of cortical stimulation was tested at 1 sec intervals during the inspiratory burst. The stimulus current was delivered in single pulses of 1 msec duration, at 1 ma and 4 ma intensity. At each site, anodal, then cathodal stimulus polarities were used. The reference electrode was located in the adjacent skin of the scalp. Histological analysis of low threhold sites was facilitated by probe lesions made at the end of the recording sessions.

The inspiratory responses to cortical stimulation were characterized by an inhibition of the firing activity when the stimulation occurred during the first half of the inspiratory busrts. The inhibition elicited during the second half was sufficient to momentarily, completely inhibit the inspiratory motor activity. When presented late in the second half of the inspiratory burst, off switching of the inspiratory activity was frequently observed to be elicited by the stimulus. Inhibition of inspiratory activity was produced at all cortical sites in the pericruciatate region using 4 ma stimulus intensity. Cathodal current produced a 12% more inhibition of the inspiratory activity than anodal current. However, at the cortical sites where the phrenic evoked primary cortical evoked potential was the largest, 1 ma stimulation produced 16 - 57% more inhibition of the inspiratory discharge than was elicited by stimulation at the other cortical sites. Low threhold sites were located in the postcruciate gyrus at the intersection of areas 3a and 4-gamma and also in the 3b cortex of the ansate gyrus.

456.7 IS THE OXYGEN-INDUCED REDUCTION IN THE RESPIRATORY FREQUENCY DEVELOPED DURING NORMOBARIC HYPEROXIA INDEPENDENT FROM CNS OXYGEN TOXICITY? D. Torbati* (SPON: S.L. Liles), Inst. For Environ. Med. Univ. of Pennsylvania, Philadelphia, PA 19104 and Dept. of Neurosurgery, LSU Sch. of Med. New Orleans, LA 70112-2822.

Brain and lungs are among the sensitive targets to the toxic effects of hyperbaric oxygenation. The respiratory system in conscious mammals is believed to be primarily affected by oxygen at pressures not exceeding 3 atmospheres absolute (ATA), while at higher pressures, the CNS oxygen toxicity precedes the respiratory effects of oxygen. In previous studies, a close correlation between changes in the regional cerebral metabolic rate for glucose (rCMRgl) and the neurological manifestations of oxygen toxicity was demonstrated in rats exposed to 3 and 5 ATA oxygen (Torbati D., et al, Brain Res. 262:267, 1983; Torbati D. and Lambertsen C.J., Brain Res., 344:186, 1985). A significant transient decrease in frequency of breathing (f) is an early characteristic sign of respiratory oxygen toxicity at pressures lower than 3 ATA oxygen (Simon A.J. and Torbati D., Undersea Biomed. Res., 9:263, 1982). In the present study, a possible interaction between central and respiratory effects of normobaric hyperoxia was tested during development of hyperoxic-induced reduction in the f of conscious rats. Alterations in rCMRgl, measured by ^{14}C 2-deoxyglucose autoradiographic technique, and the f were used as indices for the CNS and respiratory sensitivity to hyperoxia, respectively. No significant differences in rCMRgl were found between two groups of air-exposed and oxygen-exposed rats in 29 neuroanatomical structures examined, including the medullary respiratory centers, at 24 h of hyperoxia. At the same time, a significant reduction in the f was observed only in the oxygen-exposed rats. It is concluded that the initial hyperoxic-induced reduction in f is apparently independent of the CNS effects of oxygen toxicity. (Supported by ONR contract No.N00014-81 K04404).

456.8 EFFECTS OF VAGOTOMY AND HYPOXIA ON PHRENIC ACTIVITY IN NEONATAL SWINE. P.M. Gootman, H.L. Cohen, L.P. Eberle*, A.M. Steele*, B.W. Hundley*. Dept. Physiol. SUNY-Health Sci. Cent. Bklyn, Brooklyn, NY 11203 and Schneider Child. Hosp. LIJ-HMC, New Hyde Park, NY.

We have already shown that pulmonary afferent inputs can have marked effects on PHR activity in neonatal swine (Gootman, et al. Fed. Proc.46:1245, 1987). In the present report we document the effects of bilateral vagotomy on the high frequency oscillation (HFO) of phrenic (PHR) activity as well as whether vagotomy alters the response of the respiratory rhythm generator (RRG) to hypoxia. Experiments were performed on 25 piglets between <24 hrs and 43 days of age, anesthetized with Saffan (12-16 mg/kg/hr during surgery, 2-4 mg/kg/hr during data collection), paralyzed with C-10 and artificially ventilated on 100% O_2 except during hypoxia. Blood gases were monitored at 45 min intervals with a Radiometer microsystem. Monophasic recordings of left and/or right PHR activity (bandpass 10 Hz to 10kHz) were stored on analog tape along with integrated PHR activity (100 msec time constant), intratracheal pressure, aortic pressure, EKG and markers indicating phases of artificial ventilatory cycle. Two degrees of hypoxia: 21% and 10% inspired O_2 (PaO_2 45-65 and 20-30 torr, respectively) were induced by ventilating the animals with gas mixtures containing O_2 and N_2. The durations of the inspiratory (I) and expiratory (E) phases were calculated from the integrated PHR. Power spectral analyses of PHR activity were triggered during the I phase on an IBM AT computer. Power spectral densities were investigated at a sampling interval of .244 msec, 2048 data points/breath (sampling rate 4.096 kHz, yielding 2Hz/bin frequency resolution) using the Fast Fourier Transform (FFT). Two 1024 data point FFTs were computed per 500 msec epoch. The Nyquist foldover rate determined the low pass filter setting of 1 kHz. Average power was computed from 200-300 epochs. Hanning windows were applied to reduce side-lobe leakage.

The changes in duration of the I and E phases were not as marked as those reported in adult mammals. Duration of the I phase did not change significantly while the duration of the E phase either increased or remained the same. Thus overall respiratory rate did not markedly change. There was a tendency towards a shift to a lower frequency (ca. 15 Hz decrease) in the PHR HFO following bilateral vagotomy. Power spectral energy increased following vagotomy in piglets >1 wk. Hypoxia within the first 2 min tended to increase the amplitude of the HFO but did not shift its peak frequency. The duration of the E phase increased significantly during hypoxia. Bilateral vagotomy did not alter the RRG's responses to hypoxia. Thus, bilateral vagotomy had less of an effect on the RRG in neonatal swine as compared to adult mammals. (Supported by USPHS NIH grant # HL-20864. Saffan was supplied by Glaxo, Inc.)

456.9 DELAYED PROCESSING OF ACTIVE OPIOIDS IN THE NUCLEUS TRACTUS SOLITARIUS MAY EXPLAIN THE NEONATAL BIPHASIC RESPIRATORY RESPONSE TO HYPOXIA. I.R. Moss and J.G. Inman*. Dept. of Pediatrics, Univ. of Texas Health Sci. Ctr. at Dallas, Dallas, TX 75235.

As part of our long-standing interest in the relationship between neuromodulators and respiratory control, we had shown that endogenous opioids suppress breathing. In a recent study we have shown that there is a difference in responsiveness to hypoxia between very young and older piglets, in that the young, unlike the old, respond to hypoxia with transient hyperventilation followed by severe depression of breathing. Naltrexone, the opioid antagonist, reverses this so-called biphasic response to hypoxia in the younger piglets, suggesting that opioid peptides may play a role in the respiratory suppression during hypoxia. To explore this hypothesis further, we have measured levels of β-lipotropin, β-endorphin and acetylated β-endorphin in blood, cerebrospinal fluid (CSF), pituitary, cerebrum and nucleus tractus solitarius (NTS) of 1-4 day old piglets at control and during prolonged hypoxia (10% O_2/N_2 for $\geq$ 30 min). Experimental procedure included decapitation and rapid collection, processing and freezing of tissues until analysis by radioimmunoassay. The assays utilized materials from DuPont NEN (Boston, MA) and Peninsula Laboratories (Belmont, CA), including antisera against human opioid peptides and a human opioid tracer. Preliminary experiments showed good parallelism between human standard curves and porcine pituitary dilution curves using the human antisera.

Our most significant findings were those in the NTS: while β-lipotropin (the precursor) and β-endorphin (the active opioid) increased significantly during hypoxia as compared to control, acetylated β-endorphin (the inactivated peptide) did not. Thus, because of slow processing, there may be an accumulation of β-endorphin during hypoxia in young animals. This would explain the physiological diminution of the hyperventilatory response to hypoxia in neonates. Other results show that 1) the distribution and proportional response in the NTS are different from those in the pituitary, leading to the conclusion that the opioid peptides in the NTS are synthesized in situ, and 2) that the levels of endogenous opioid peptides in the NTS, which is an important nucleus for respiratory control, can not be deduced directly from measurements of these peptides in more accessible body compartments, such as CSF and blood. (Supported by NIH NHLBI RO1 HL36939)

456.10 AGE RELATED DIFFERENCES IN BRAINSTEM RESPIRATORY CHEMOSENSITIVE NEURONAL ACTIVITY: J.A. Whittaker, Y. Pan, D.G. Bernard, P.W. Archer, S. Merrill, C.O. Trouth, Dept.Physiol & Biophysics, Coll. Med., Howard University., Washington, D.C. 20059.

Three chemosensitive areas on the ventrolateral medullary surface (VMS) are involved in the central chemical CO_2 regulation of respiration; a rostral(Area-M) an intermediate(Area-S) and a caudal (Area-L). The resent investigation examines the hypothesis that the central CO_2-chemosensory apparatus, might from a functional point of view not be fully developed at birth. This project utilizes the cat as an animal model for the study of age-related differences in the central chemosensory mechanism in normal neonatal(age 1-5 days) postnatal(age,6-8 weeks) and adult (age 7 months)animals. In spontaneously breathing animals anesthetized with chloralose-urethane, neurons in the caudal chemosensitive area were tested for their responsiveness to increased inspired CO_2($PICO_2$),decreased inspired O_2(PIO_2)mock C.S.F.-pH changes(7.4.-control;7.0-acid;7.8 alkaline), topically applied acetylcholine(ACh),Morphine,(MOR)and Naloxone (NAL). Topical application was by means of filter paper pledgets soaked in the test drugs that were prepared in mock CSF-pH 7.4. Units were also tested for their responsiveness to other modalities of sensation such as touch,pressure, joint manipulation and temperature. In the ADULT and POSTNATAL animal, non-phasic pH sensitive units increased their firing rate in response to increased $PICO_2$ (5% and 7%),acid CSF,ACh and the opiate antagonist NAL,whereas Alkaline CSF and MOR decreased firing rate. NAL reversed the depressant effects of MOR and alkaline CSF and potentiated the effects of increased $PICO_2$,ACh, and acid CSF. In the NEWBORN, increased $PICO_2$ and ACh caused slight increase to no changes in neuronal activity, whereas MOR and decreased PIO_2 caused marked increase in activity. Some units fired in bursts, not synchronous with respiration,however interspersed with periods of no activity or smaller amplitude units. During decreased PIO_2 sitmulation, larger amplitude spikes with irregular firing pattern appeared among the bursts. Support: NIH-MBRS 2-S06RR08016-16 and PHS-5-T32-GM07800.

456.11 ANTICHOLINESTERASE TOXICITY AT BRAINSTEM RESPIRATORY CHEMOSENSITIVE AREA: P.W. Archer, Y. Pan, J.A. Whittaker, D.G. Bernard, R.M. Millis, C.O. Trouth,(SPON: J.A.Holloway),Dept.Physiol & Biophysics, Coll.Med,Howard University,Washington,D.C. 20059.

Failure of the respiratory control mechanism in the central nervous system(CNS)has been suggested as the dominant factor in the lethality of organophosphate(OP)anticholinesterase(anti-AchE)nerve agents. Pyridostigmine(PYR)and physostigmine(PHY)have been proposed as pretreatment agents in organophosphate intoxication however, since PHY and PYR are also anticholinesterase agents there is great potential for these drugs to depress ventilation. In the present study the respiratory effects of cholinomimetics applied topically to the three respiratory chemosensitive areas(Areas M,S and L)on the ventrolateral medullary surface(VMS)were investigated in chloralose urethane anesthetized cats. Topical application was by means of filter paper pledgets($2mm^2$)soaked in test drugs at various concentrations and prepared in mock cerebrospinal fluid(CSF)at pH 7.4. Low concentrations of Acetylcholine(ACh)2-25 mg% resulted in graded increases in Tidal volume(V_T)and frequency(f)in Areas M,S and L without cardiovascular effects at Areas-S and L. Topical ACh at higher concentrations(>200mg%)caused transient increase in MABP at Area-M followed by hypotension. Nicotine applied topically(2-20mg%) increased V_T and f in three chemosensitive areas whereas mean arterial blood pressure(MABP)decreased significantly in Areas S and L and increased in Area M. These effects were not antagonized by Hexamethonium. Intravenous injections of Nicotine increased V_T, f and MABP. Pyridostigmine(PYR)and Physostigmine(PHY)applied topically at low doses(2-20mg%)to areas M, S and L increased respiration without cardiovascular effects. At higher concentrations($\geq$400mg)PHY and PYR applied to Area L produced respitory arrest in the end-expiratory position(apnea)followed by marked decrease in MABP. Atropine sulfate(AS)8-25mg% reversed the PYR and PYR induced hypotension but apnea persisted. Naloxone(2-8mg%)and Theophyllin(50mg%)reversed the apnea. At Area-S,similar doses of PHY and PYR produced decrease in MABP followed after 3 mins.by a gradual decrease in V_T to apnea. Time constants of V_T changes were significantly greater at Area-S than Area-L. Neither AS,NAL nor THEO reversed the apnea and MABP effects of Area-S. At Area-M, decreases in V_T were followed by decreases in MABP;both effects were reversed by AS and THEO. These effects were comparatively small and did not include apnea. In summary: The cat's VMS consists of regions of differential sensitivity to the carbamates PHY and PYR(sensitivity in Areas L>S>M).At Area S,respiratory responses appeared secondary to cardiovascular effects;whereas in Areas M and L,respiratory responses occurred simultaneously with cardiovascular responses. Antagonism to apnea and hypotension at Area-S may involve other mechanisms. Supported by: NIH-MBRS 2-S06 RR08016-16 and PHS-5-T32 G-M07800.

456.12 EFFECT OF GLYCINE AND NICOTINE AT BRAINSTEM RESPIRATORY CHEMOSENSITIVE AREAS: R.M. Millis, P.W. Archer, Y. Pan, J.A. Whittaker, D.G. Bernard, C.O. Trouth, (SPON: G-M. Moolenaar), Dept. Physiol. & Biophysics, College of Medicine Howard University.,Washington,D.C. 20059.

The ventral medullary surface (VMS) contains a number of pharmacologically sensitive regions which influence different body functions. Neuronal Sensing elements involved in the central chemical regulation of breathing have been described within the marginal glial layer. Glycine sensitive areas near the trapezoid bodies and Nicotine sensitive areas medial to Nerve XII rootlets are involved in cardiovascular regulation. The purpose of this study was to determine the effect of Nicotine and Glycine(which were effective in demarcating the VMS areas involved in cardiovascular regulation)on the three respiratory chemosensitive areas(M,S,&L) in chloralose-urethane anesthetized adult cats. The drugs were applied topically by means of filter paper pledgets ($1mm^2$)soaked in the test drugs at various concentrations and prepared in mock cerebrospinal fluid (CSF) at pH 7.4. In the caudal Area(Area L) low concentrations of Glycine (2-20mg%)produced no significant respiratory or cardiovascular changes. At 100mg% Glycine decreased mean arterial blood pressure (MABP),Tidal Volume(V_T),respiratory frequency(f)and respiratory minute ventilation($\dot{V}_E$)whereas 125mg% Glycine produced apnea and marked hypotension. The respiratory and cardiovascular depressant effects of Glycine were reversed by Strychrine (1 mg%) applied topically to Area L. At higher concentrations (>5mg%)Strychnine induced convulsions. Less marked responses were obtained from the rostral area. In the Intermediate area-(Area-S),Glycine caused respiratory depression and hypotension only at higher concentrations ($\geq$ 400mg%).

In the caudal Area topical application of Nicotine produced significant ($p<0.001$)increases in V_T, f and $\dot{V}_E$ whereas MABP decreased significantly. Increase in V_T and f started with the first breath following Nicotine application. Intravenous injections of 200ug Nicotine increased V_T, f, $\dot{V}_E$ and MABP. Topical application of hexamethonium (1mg/ml)only partially antagonized the effects of Nicotine. The effect of Nicotine in Intermediate Area was less marked causing increased V_T, f, $\dot{V}_E$ and decreased MABP. These effects were not antagonized by hexamethonium. Nicotine however caused increases in V_T f, $\dot{V}_E$ and MABP in the rostral area. Conclusion: 1.The effect of Nicotine on Areas L and S appears to be local,whereas the effect following iv administration appeared to be via the general circulation. 2.The effect of glycine at the caudal and intermediate areas do not support the findings of other workers. Supported by: NIH-MBRS 2-S06 RR08016-16 and PHS-5-T 32GM07800.

457.1 REGULATION OF DOPAMINE (DA) SYNTHESIS IN STRIATAL SLICES: A MODEL FOR DA AUTORECEPTORS, E.A. Novak*, R.S. Salah, D.Clark*, and M.P. Galloway, (SPON: E. Schoener), Lafayette Clinic, Sinai Hospital, and Wayne State Univ. Sch. Med., Detroit, MI 48207

In order to examine the molecular mechanisms of dopamine (DA) autoreceptors, we have further developed the brain slice model of Wolf et al. (1986) to measure autoreceptor (AR) modulation of tyrosine hydroxylation in striatal slices. Historically, synthesis modulating ARs have been defined in vivo utilizing the GBL model of Walters and Roth (1976). In light of this, we have compared our results obtained in the slice to previous pharmacological descriptors obtained in vivo.

In the slice, DOPA accumulation (after inhibition of decarboxylase with NSD-1015) is linear with protein and time, temperature dependent, and increased 50-100% in the presence of 30 mM K^+. After incubation for 30 min with NSD, 90% of total DA is intracellular, 90% of total DOPAC extracellular, and DOPA is evenly distributed between the the two compartments. Thirty mM K^+ induces DA release and increases the extracellular concentration of DA from approximately 30 nM to 300 nM. Under basal conditions, DA synthesis is inhibited by the following DA agonists (EC50 in nM, maximum % inhibition): apomorphine (145nM, 90%), DA (620nM, 90%), quinpirole (140nM, 45%), pergolide (80nM, 40%), BHT920 (80nM, 30%), 7-OHDPAT (100nM, 50%), (+)-3PPP (1800nM, 70%). The D2 anatagonist sulpiride, but not the D1 antagonist SCH 23390 or the reuptake blocker nomifensine, blocked the effect of APO (100 nM). In addition, sulpiride shifted the dose response curve of quinpirole to the right suggesting a receptor mediated inhibition of DA synthesis. To address the issue of variable efficacies in this preparation, slices were exposed to 30 mM K to activate TH and thus simulate conditions observed in vivo after GBL. Under these circumstances, agonists such as quinpirole and pergolide fully reversed DOPA accumulation to levels below K^+ control. Interestingly, D1 selective agonists such as SKF 38393 (430 nM, 90%) and fenoldopam (1000 nM, 90%) also decreased DA synthesis. The effect of SKF 38393 was not attenuated in the presence of nomifensine, sulpiride or SCH 23390. The inhibition of DA synthesis by quinpirole (3 uM) was potentiated in the presence of SKF 38393, suggesting separate sites of action for these two compounds.

Together the data suggest that 1) autoreceptor regulation of DA synthesis in our slice preparation parallels the in vivo model, and 2) issues of intrinsic agonist efficacy, D1-D2 receptor character, and receptor-function coupling can be evaluated in a physiological, yet defined, milieu. Supported by NIMH 41227, NIDA 04120, UPF, PMAF, and the State of Michigan-DMH.

457.2 EFFECTS OF cAMP ELEVATING AGENTS AND DOPAMINE AUTORECEPTOR AGONISTS ON PHOSPHORYLATION AND ACTIVITY OF TYROSINE HYDROXYLASE IN STRIATAL SLICES, R.S. Salah, D.M. Kuhn, and M.P. Galloway, Depts. of Pharmacology and Psychiatry, Wayne State Univ. Sch. of Med. and Lafayette Clinic, Detroit, MI 48207.

To investigate a role for protein phosphorylation as a mechanism for modulation of net tyrosine hydroxylase (TH) activity in situ, the incorporation of radiolabelled phosphate into striatal slices was investigated. Two dimensional slices (0.3 mm x 0.3 mm) were prepared and maintained in a physiological buffer for a total of two hours in the presence of inorganic ^{32}P (approximately 1 mCi/ mg protein). Autoradiography was used to assess phosphate incorporation into total striatal proteins (SDS-PAGE), and into TH isolated by immunoprecipitation (rabbit polyclonal antibody directed against PC-12 TH).

These incubation conditions resulted in the successful incorporation of ^{32}P into numerous striatal proteins including TH. The level of basal phosphorylation was compared to phosphorylation in the presence of activators of TH and/or dopamine (DA) autoreceptor agonists. Forskolin (10 uM), and dibutyryl cAMP (1 mM) each resulted in a 50-100% increase in TH activity, measured as DOPA accumulation in the presence of NSD-1015. These same agents also induced an increase in incorporation of ^{32}P into TH and other striatal proteins. Preliminary experiments with the DA autoreceptor agonist quinpirole have failed to demonstrate a decreased incorporation of phosphate into TH, even though this drug reduces basal DA synthesis rates in striatal slices by 40-45%. On the other hand, when slices were incubated in the presence of quinpirole and one of the TH activating agents described above, quinpirole was able to decrease phosphorylation below the activated control level.

These studies suggest that the incorporation of phosphate into striatal TH can be investigated in situ through the use of brain slices. Furthermore, the finding that DA agonists can attenuate the increased phosphorylation of TH brought about by agents which raise cAMP levels is consistent with the hypothesis that autoreceptors are negatively coupled to adenylate cyclase. The ability to immunoprecipitate phosphorylated TH, measure DA synthesis rates, and manipulate DA autoreceptor function in the same preparation affords a unique opportunity for studying autoreceptor transduction processes. Supported by DA-0444120, MH-41227, the State of Michigan-DMH, and WSU Neuroscience Program.

457.3 SYNERGISTIC EFFECTS OF DOPAMINE D1 AND D2 AGONISTS ON ROTATION IN RATS LESIONED UNILATERALLY WITH 6-HYDROXYDOPAMINE: ANATOMICAL SITES OF ACTION. H. A. Robertson and G. S. Robertson, Department of Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

At the molecular level, it appears that D1 and D2 receptor agonists have opposite effects. For example, D1 agonists increase release and D2 agonists decrease release of cyclic AMP from striatal slices (Stoof, J.C. and Kebabian, J.W. Nature, 294: 366, 1981). However, D1 and D2 agonists have synergistic effects on turning when tested on rats unilaterally lesioned with 6-hydroxydopamine (6-OHDA) (Robertson, G.S. and Robertson, H.A. Brain Res., 84: 387, 1986). We have proposed that the resolution of this paradox may lie in the anatomical location of the D1 and D2 sites involved in turning behaviour. Our hypothesis is that animals with unilateral 6-OHDA lesions turn as a result of activation of D1 dopamine receptors in the substantia nigra and D2 dopamine receptors in the striatum. This hypothesis is supported by electrophysiological and biochemical evidence. This hypothesis explains, among other things, the efficacy of the combination of L-dopa and bromocriptine in Parkinson's disease (see Robertson, H.A. and Robertson, G.S., Clin. Neuropharmacol., in press).

To test this hypothesis we have studied the effects of a selective D1 agonist SKF 38393 and the selective D2 agonists LY 171555. These selective agonists were administered either systemically or via injection directly into either the substantia nigra or the striatum in rats lesioned unilaterally with 6-OHDA. Systemic injections of doses of the two agonists (SKF 38393 and LY 171555), which alone produce little or no turning, will in combination produce rapid circling away from the lesioned side. Similarly, injections of SKF 38393 directly into the substantia nigra followed by an injection of LY 171555 given directly into the striatum resulted in an additive effect on circling behaviour.

These results, together other findings, suggest that circling behaviour in 6-OHDA lesioned rats depends on activation of D1 dopamine receptors in substantia nigra and D2 dopamine receptors in striatum. These results suggest that careful titration of D1/D2 stimulation by selective agonists may be useful in treating Parkinson's disease.

We thank the Parkinson Society of Halifax, the Parkinson Foundation of Canada and the Medical Research council of Canada for support.

457.4 L-DOPA INCREASES DOPAMINE SYNTHESIS IN THE 6-HYDROXYDOPAMINE-LESIONED SUBSTANTIA NIGRA: CORRELATION WITH ROTATIONAL BEHAVIOUR.
G. S. Robertson and H. A. Robertson, Department of Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

Rats lesioned unilaterally by infusion of 6-hydroxydopamine (6-OHDA) into the substantia nigra pars compacta (SNpC) rotate towards the side contralateral to the lesion after systemic L-dopa injection. The contralateral direction of rotation suggests that L-dopa-induced turning is mediated by the lesioned side. Dopamine (DA) can be released from both the terminal fields of the nigrostriatal pathway which innervate the striatum and from the dendrites of these neurons which branch into the substantia nigra pars reticulata. In order to determine the degree to which SNpC lesioning with 6-OHDA alters DA synthesis in these sites the following study was undertaken.

Male Sprague-Dawley rats were lesioned by injecting 8ug 6-OHDA in 4ul of saline unilaterally into the SNpC. After at least 4 weeks, the success of the 6-OHDA lesion was assessed by determining the rotation rate to an apomorphine (0.5 mg/kg, i.p.) challenge. Rats which displayed rotation rates of at least 7 turns/minute were used in the remainder of the study. These animals were then subjected to either the experimental condition, carbidopa (25 mg/kg, i.p.) followed 1 hour later by L-dopa (25 mg/kg, i.p.) or two injections of the vehicle. One hour after the last injection the animals were sacrificed and the striatum and the substantia nigra (SN) from both the lesioned and the intact side were rapidly dissected on ice. The tissue samples were stored at -70 degrees centigrade in 0.1N PCA until determination of DA and DOPAC levels by HPLC.

Animals which rotated at a rate of at least 7 turns/minute during the apomorphine test had DA and DOPAC levels that were reduced by 98% in the denervated striatum. The DA and DOPAC content of the lesioned SN was reduced by half in these animals. L-dopa increased the amount of DA and DOPAC on the lesioned side in both the SN and striatum. Interestingly, L-dopa increased the lesioned SN content of both DA and DOPAC by 10 fold relative to the corresponding SN in vehicle treated rats. In contrast the striatal content of both DA and DOPAC was increased by only 4 times in the denervated side after L-dopa treatment relative to the same side in the vehicle controls. These results suggest that L-dopa induced turning is mediated by an increase in DA both the striatum and SN on the lesioned side.

We thank the Medical Research council of Canada and the Parkinson Foundation of Canada for support.

457.5 STUDIES ON THE IN VIVO CATABOLISM OF EXOGENOUS DA AS INFUSED THROUGH A PUSH-PULL CANNULA IMPLANTED IN THE RAT CAUDATE NUCLEUS. G.D. Chang and V.D. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

Determination of the levels of dopamine (DA) metabolites in the brain has provided pivotal information regarding how central acting agents alter DAergic activities and DA metabolism. Recently two important techniques have been widely used to measure in vivo efflux of endogenous neurochemicals from discrete brain areas; intracerebral dialysis and push-pull perfusion techniques. Coupled to HPLC with electrochemical detection, these techniques allow the measurement of endogenous monoamines and monoamine metabolites simultaneously and more importantly allow continuous sampling in freely behaving animals. In the present study, we have adopted the push-pull perfusion and HPLC-EC technique to study the effects of exogenous DA on the output of neurochemical substances from the caudate nucleus (CN) of freely behaving rats.

Push-pull cannulae of 22 gauge were aimed at the CN of adult male rats according to the deGroot's atlas. Ten to twenty days after implantations, modified Krebs-Ringer phosphate medium (KRP), pH 7.4, was infused through the inner cannula at a flow rate of about 12 µl/min. Perfusates of 15 min intervals were subjected to HPLC-EC analysis of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA). Six animals received local infusions of 10^{-5}M, and 10^{-4}M DA in KRP for 15 min at 1400h and 1530h, respectively. Another four rats received two infusions of $5x10^{-5}$M DA in KRP at 1400h and 1600h, each for 15 min. However 30 min after the first infusion of $5x10^{-5}$M DA, 10^{-3}M Nomifensine was included in the KRP till the end of perfusion.

Infusions of exogenous DA at these three concentrations elicited significant increases in DOPAC output from all animals. The total amounts of DOPAC increases induced by 10^{-5}M, $5x10^{-5}$M and 10^{-4}M DA were 1097±213, 2674±716, and 5354±685 pg, respectively. In addition, the higher two doses of exogenous DA also induced increases in HVA output. The increases in DOPAC output by $5x10^{-5}$M DA was reduced by 60% by the inclusion of 10^{-3}M Nomifensine in KRP. Interestingly exogenous DA-induced increases in HVA output were little affected by the Nomifensine treatment. Furthermore, infusions of exogenous DA did not change 5-HIAA output. In conclusion, our results confirm in vivo that (a) DA catabolic pathway via DOPAC intermediate predominates over the alternative pathway via 3-methoxytyramine and (b) increases in extracellular DA levels, which mimic increases in DA release, will lead to increases in DOPAC and HVA levels in extracellular space. In addition, the infusions of exogenous DA do not appear to affect serotoninergic activities.

457.6 L-DOPA-INDUCED INCREASE IN DA RELEASE FROM STRIATUM: IN VITRO STUDIES ON MECHANISM OF ACTION G.L. Snyder and M.J. Zigmond. Department of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

L-Dihydroxyphenylacetic acid (DOPA) is effective in reversing the neurological deficits caused by nigrostriatal bundle degeneration, apparently by increasing the availability of dopamine (DA) in brain. The current experiments examine the location at which exogenous DOPA is converted to DA and the mechanism by which the newly synthesized DA is released. In addition we have assessed the effects of supplementation with tyrosine, the amino acid precursor of DA.

Striatal slices (350 uM) were continuously superfused (100 ul/min) with oxygenated Krebs buffer at 37°C. DOPA (10 uM) or tyrosine (150 uM) were added to the superfusion buffer and 60 min later slices were exposed to electrical field depolarization (10 mAmp, bipolar pulses) delivered at 4.5 Hz for 15 minutes. Superfusates and tissue slices were analyzed for endogenous DA content by HPLC with electrochemical detection. Data were expressed as DA overflow (stimulated minus basal DA efflux) in ng per mg protein.

Superfusion with DOPA for 120 min produced a significant elevation in the DA content of striatal slices (basal DA content: 68.6 ng/mg; increase due to DOPA: +21.6 ng/mg) as well as a large increase in both spontaneous efflux and depolarization-induced outflow (23- and 10-fold, respectively). In contrast, tyrosine had no effect on the amount of DA found in tissue or on DA efflux. The increment in tissue DA produced by DOPA was not affected by pretreating animals with 6-hydroxydopamine (6-HDA, 200 ug, ivt.), despite the apparent destruction of 93% of the dopaminergic terminals in striatum (basal DA content: 4.9 ng/mg; increase due to DOPA: +27.4 ng/mg). However, 6-HDA reduced by 83% the increase in stimulation-evoked overflow of DA normally produced by DOPA. In order to determine whether DA overflow in the presence of DOPA was Ca^{2+}-dependent, intact striatal slices were stimulated during superfusion with a Ca^{2+}-free medium containing EGTA (0.1 mM). Whereas the stimulation-evoked overflow of DA from untreated control slices was abolished in Ca^{2+}-free medium (-97%), the increase in DA overflow induced by DOPA was only partially reduced (-36%). These data are consistent with our previous findings on the in vivo and in vitro release of DA during exposure to DOPA (Keller et al., Soc. Neurosci. Abst. 1986). Furthermore, they suggest that the conversion of DOPA to releasable DA occurs largely in DA-containing terminals and that release occurs by both Ca^{2+}-dependent and Ca^{2+}-independent mechanisms. (Supported in part by USPHS grant NS19608.)

457.7 EXAMINATION OF DOPAMINE RELEASE FROM EXPLANTED NIGROSTRIATAL AND MESOACCUMBENS (SOMA-TO-TERMINAL) NEURONS OF THE ADULT RAT BRAIN. M.D. Davis* and C.D. Kilts* (SPON: C. Phifer). Dept. Psychiatry, Box 3870, Duke Univ. Medical Center, Durham, North Carolina 27710.

The physiology and pathology of dopamine (DA) neurotransmission in the CNS is increasingly recognized to represent the expression of the function and dysfunction of critical subsets of DA neuronal populations. Consistent with this organization, optimal pharmacotherapy would reflect DA neuronal population-selective drug actions. However, empirical approaches to the delineation and study of DA neuronal systems have been hindered by the confounding, uncontrolled influences characteristic of in vivo studies and the uncertain relevance of in vitro brain slice preparations. As a fruitful comprimise, we have attempted to isolate and study two distinct DA neuronal population explants containing the anatomically and functionally continuous cell body-to-terminal relationship.

Adult male Sprague-Dawley rats were decapitated, their brains were removed and two coronal cuts were made, the first at the level of the rostral head on the caudate nucleus, the second just anterior to the pons. A sharpened stainless-steel cannula (0.6 mm, i.d.) attached to a mechanical vibrator was guided through the tissue block by a predetermined route to collect cell bodies at the lateral edge of the ventral tegmentum (A-10, for the mesoaccumbens projection) or the substantia nigra pars compacta (A-9, for the nigrostriatal projection), their axons running through the dorsal aspect of the medial forebrain bundle and their terminal fields in the nucleus accumbens or caudate putamen, respectively. The cylindrical explant was anchored to the wax bottom of a perifusion chamber. The rostral 3 mm was inserted into a 1.0 mm diameter plastic collection tube with which the incubation medium bathing the nerve terminals was drawn off via a peristaltic pump. Samples were collected over an 8 minute period and then analyzed by HPLC-EC for dopamine release. The integrity of the soma-to-terminal dopamine projections in each explant was verified by physiological, pharmacological and histological methods.

Electrical field stimulation (1 - 30 Hz) of the cell body region of mesoaccumbens or nigrostriatal explants elicited a frequency-dependent increase in dopamine release from the nucleus accumbens or caudate putamen. Neurotensin (0.001 - 0.1 uM) applied continuously to the perifusion media induced a concentration-dependent elevation (50 - 250%) in dopamine efflux which was augmented by co-application of the dopamine receptor blocker, haloperidol (10 nM). These effects were blocked by explant transection, indicating the involvement of somato-dendritic as opposed to terminally-localized neurotensin receptors. Thus, we have demonstrated viability, functional continuity and utility of these explants and are continuing to investigate the neuromodulatory influence of other putative peptide and non-peptide neurotransmitters on this model.

457.8 SIMULTANEOUS IN VIVO MEASUREMENTS OF DOPAMINE RELEASE AND METABOLISM IN THE RAT MEDIAL PREFRONTAL CORTEX AND STRIATUM USING INTRACEREBRAL DIALYSIS. L.E. Goldstein, C.W. Bradberry, R.H. Roth and B.S. Bunney. Yale Univ. Sch. Med., New Haven CT 06510.

The mesoprefrontal cortical dopamine (DA) system appears to have a different biochemical, pharmacological and electrophysiological profile than that observed in the nigrostriatal DA system (Roth, R. H., Ann. NY Acad. Sci., 430:27, 1986). The purpose of the present study is to examine in vivo neurotransmitter release, regulation and metabolism within the terminal fields of the medial prefrontal cortex (mPFC) and striatum of the rat. Using in vivo dialysis coupled to a high resolution liquid chromatography system, we are able to monitor extracellular levels of DA and the DA metabolites, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC), as well as the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) from both terminal fields.

Improved dialysis probes, with recoveries of 25-35% for DA and metabolites, are constructed from 300 um O.D. cellulose acetate dialysis tubing and implanted dorsoventrally in all animals. Two animal preparations are used: (1) acute anesthetized rats (chloral hydrate or halothane), and (2) chronically-implanted, unanesthetized freely moving rats. Dialysate volumes of 25 ul are collected over 25 min and analyzed by a liquid chromatography-electrochemical detection system equipped with a microbore column. The limit of detection of DA and metabolites in the recovered dialysate samples is routinely between one and five femtomoles.

Our results lead us to the preliminary identification of DA, DOPAC, HVA, and 5-HIAA in dialysates obtained from the mPFC and striatum of both animal preparations. Chromatographic peaks corresponding to DA and metabolites obtained from terminal field dialysate samples show identical retention times to authentic reference compounds under varying chromatographic conditions. Furthermore, administration of pharmacological agents known to alter DA release and metabolism reveal the anticipated responses: (1) alpha-methyl-p-tyrosine results in a significant decrease in the levels of DA and its metabolites, (2) d-amphetamine significantly increases the levels of DA while the levels of the DA metabolites decline, (3) reserpine induces a significant increase in DA and metabolite levels, (4) 6-OHDA lesions of the mPFC lead to profound depression in both DA and DA metabolite levels, and (5) the anxiogenic beta-carboline FG 7142 increases DA and DA metabolite levels.

We are currently investigating the effect of intravenous administration and local application of the D-1 and D-2 agonists (i.e., SKF 38393 and LY 171555), and D-1 and D-2 antagonists (i.e., SCH 23390 and sulpiride) on DA and metabolite levels. Our preliminary results suggest that the D-2 agonist decreases and D-2 antagonist increases DA levels. USPHS grants MH-28849 and MH-14092.

457.9 STRIATAL EXTRACELLULAR DOPAMINE LEVELS IN CONSCIOUS RATS DURING NORMAL LIGHT-DARK CYCLES AND GLOBAL CEREBRAL ISCHEMIA.
J. A. Clemens, L. A. Phebus*, W. L. Russell* and D. P. Henry*. The Lilly Research Laboratories, Eli Lilly and Company, 46285. Dept. of Pharmacology, Indiana Univ. Sch. Medicine, Indianapolis, IN 46202.

Extracellular dopamine levels were measured using in vivo brain microdialysis and radioenzymatic or HPLC-EC assays. The dopamine metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) as well as the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) were measured in striatal dialysate by HPLC-EC.

Three days after surgical implantation of the dialysis probe into the striatum of Sprague-Dawley rats, dialysate samples were collected at three hour intervals and assayed for dopamine levels by radioenzymatic assay. Physiological Ringers solution was perfused at a rate of 1.0 µl per minute. Extracellular dopamine concentrations increased at the onset of the dark period (630 +/- 122 to 1156 +/- 156 picograms/ml, N=6), remained elevated during the dark (mean = 1046 +/- 81, N=24), decreased at the onset of the light period (922 +/- 124 to 678 +/- 107, N=6) and remained low during the light period (mean = 707 +/- 58, N=24). These results indicate a diurnal rhythms exists for striatal dopamine release.

In another experiment, striatal dialysate samples were collected before, during and after 30 minutes of global cerebral ischemia induced by the four vessel occlusion technique in Wistar rats. Rats were implanted with dialysis probes 24 hours before being subjected to ischemia. Ringers solution was perfused through the probe at a rate of 1 microliter/minute and ten-minute samples were collected. Samples were assayed for dopamine, DOPAC, HVA and 5-HIAA by HPLC-EC. Dopamine levels were below detection limits (<0.5 nanograms/ml) in pre-ischemia samples. During ischemia, striatal extracellular dopamine levels rapidly increased to 174 +/- 74 nanograms/ml. On reperfusion, dialysate dopamine levels soon dropped below detectable levels. DOPAC, HVA and 5-HIAA levels decreased during ischemia. DOPAC and HVA levels increased during reperfusion.

457.10 HISTAMINE-EVOKED RELEASE OF NOREPINEPHRINE FROM RAT HYPOTHALAMIC SLICES.
P. Blandina*, P.J. Knott, L.K.H. Leung*, J.G. Young and J.P. Green Departments of Pharmacology and Psychiatry, Mount Sinai School of Medicine, City University of New York, New York, NY 10029

All evidence supports the hypothesis that histamine is a neuroregulator or neuromodulator in brain (Prell and Green, Ann. Rev. Neurosci. 1986, 9: 209). Among the roles attributed to histamine is the hypothalamic control of release of prolactin and other peptides. The hypothalamus is rich in histamine, its metabolites, the enzymes that form and metabolize histamine, histaminergenic nerve endings, and its turnover of histamine is higher than that of any other brain region. The role of histamine in the release of endogenous norepinephrine (NE) from superfused hypothalamic slices was assessed. Eight week old, male Sprague-Dawley rats were given pargyline, 75mg/kg, i.p., to inhibit monoamine oxidase. The slices were incubated in an artificial medium containing 20 uM cocaine. Measurements were made by high performance liquid chromatography with electrochemical detection. Spontaneous release of NE from the slices average 322 ± 69 fmol/mg of protein/3 min. Superfusion with histamine (10^{-5}M) or the H_2 agonist dimaprit (10^{-5}M) resulted in a 1.5 fold elevation of NE release which promptly returned to basal levels when the agonists were withdrawn from the medium. Impromidine ($2x10^{-6}$M), another H_2 agonist, increased release of NE by 60%. Tiotidine (10^{-6}M), an H_2 antagonist, did not alter the spontaneous release of NE but blocked the effect of dimaprit. Homovanilic acid was not detected in the superfusates of slices from pargyline treated animals. These data imply that histamine functions in the release of NE from the rat hypothalamus by activating the H_2 receptor. Supported by a grant (MH31805) from the National Institute of Mental Health.

457.11 ACUTE EFFECTS OF AMPHETAMINE AND COCAINE ON CATECHOLAMINE RELEASE FROM STRIATAL AND HYPOTHALAMIC REGIONS.
B.A. Bennett, M. Morris*, and D.K. Sundberg*, Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

The tuberoinfundibular neurons of the hypothalamus differ from those found in the striatum in that they have much shorter axons and do not appear to possess either presynaptic (D-2) autoreceptors or a high affinity amine uptake system. In the following experiments two stimulant drugs were used to induce release or inhibit uptake of the catecholamines, norepinephrine (NE) and dopamine (DA). This study was performed to demonstrate in vitro the differences between catecholamine regulation in these two independent dopaminergic areas.

Striatal and hypothalamic areas were obtained from Sprague Dawley male rats (250-300g). Tissues were pre-incubated in Earles, pH 7.4 in 95% O_2/5% CO_2 at 37°C for one hour. Four subsequent one hour incubation periods were conducted from the following protocol: control, experimental (drugs at 10^{-3}M), control, and followed by a 50 mM potassium. Media and tissue samples were acidified and catecholamines were alumina extracted. Catecholamine concentrations were obtained using HPLC with electrochemical detection. Shown in the table are media levels of DA and NE expressed as percent increase over control periods (n=4, *not significantly different from control).

	DOPAMINE			NOREPINEPHRINE		
	AMPH	COC	K+	AMPH	COC	K+
Hyp.	360	120*	200	320	210	750
Str.	200	270	---	160	200	110*

Amphetamine causes the release of NE and DA in both areas. On the other hand, cocaine augments media levels of dopamine in the striatum only while NE levels are increased in both areas. This data would indicate that cocaine inhibits NE uptake in both areas, whereas dopamine is inhibited in the striatum and not in the hypothalamus, as would be expected if there were no high affinity amine uptake pumps present. The potassium period was included to show the magnitude of catecholamine release resulting from nerve depolarization and suggests that multineuronal interactions are occurring particularly in regards to NE release in the hypothalamus.
This work was supported by NIH grants NS-22492 and NS-24723.

457.12 THE POSSIBLE INVOLVEMENT OF INOSITOL PHOSPHATES IN THE REGULATION OF Ca^{2+}-EVOKED RELEASE OF ^{3}H-DOPAMINE FROM SYNAPTOSOMES.
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In superfused striatal synaptosomes previously unexposed to Ca^{2+} during isolation and superfusion, 1.25 mM Ca^{2+} evokes the release of ^{3}H-dopamine (^{3}H-DA). This Ca^{2+}-evoked release was produced without the elevation of K^+ concentration (6.0 mM or less) subsequent to or during Ca^{2+} exposure, and could be modulated by DA (D2) agonists and antagonists. We now present evidence that inositol phosphates may play a role in the Ca^{2+}-evoked release of ^{3}H-DA. The concentrations of Li^+ (9 and 12 mM) necessary to partially reverse LY-171555 (a DA D2 agonist) inhibition of Ca^{2+}-evoked release indicate that this reversal may be the result of blockade of D-inositol-1-phosphate breakdown by Li^+. Concentrations of 6, 9, or 12 mM Li^+ alone were capable of significantly elevating the Ca^{2+}-evoked release of ^{3}H-DA 20-30% over control. However, 9 and 12 mM Li^+ in combination with 0.1 µM LY-171555 caused a much greater increase in the Ca^{2+}-evoked release over LY-171555 alone. For example, 12 mM Li^+ in combination with LY-171555 increased release to 114% control from the 57.1% control release produced by LY-171555 alone. Exposure of striatal synaptosomes to 9 or 12 mM Li^+ 10 min prior to Ca^{2+}-evoked release reduced 0.1 µM LY-171555 inhibition of ^{3}H-DA release from 43.9 ± 2.8% n=25 to 25.6 ± 5.8% n=16 and 22.2 ± 2.9% n=17 respectively. In olfactory tubercle synaptosomes LY-171555 inhibition was reduced from 46.3 ± 2.0% n=22 to 33.0 ± 2.8% n=18 by 9 mM Li^+ and 30.4 ± 1.9% n=22 with 12 mM Li^+. 3 mM Li^+ had no effect and 6 mM Li^+ caused a slight reduction (11.6 ± 5.7% n=16 in striatal synaptosomes) in 0.1 µM LY-171555 inhibition of ^{3}H-DA release. Concentrations of 1 mM neomycin or less have been reported to inhibit the generation of inositol 1,4,5-triphosphate (IP_3) from phosphatidylinositol 4,5-bis-phosphate. IP_3 may play an important role in Ca^{2+}-evoked release since in striatal synaptosomes 1 mM neomycin was capable of inhibiting Ca^{2+}-evoked ^{3}H-DA release by 66.8 ± 8.9% n=14 when it was present 10 min prior to Ca^{2+} exposure. In olfactory tubercle synaptosomes neomycin inhibited the release by 56.3 ± 8% n=16. Phospholipid/Ca^{2+}-dependent protein kinase is probably not involved in the Ca^{2+}-evoked release of ^{3}H-DA since 4β-phorbol 12β-myristate 13α-acetate had no significant effect on release.

457.13 CHARACTERIZATION OF THE VIP-INDUCED PHOSPHORYLATION AND ACTIVATION OF TYROSINE HYDROXYLASE IN BOVINE ADRENAL CHROMAFFIN CELLS. G.L. Craviso, J.C. Waymire, K. Lickteig*, C. Baldwin* and R.E. Zigmond. Dept. of Neurobiology and Anatomy, Univ. of Texas Med. Sch., Houston, TX 77225 and Dept. of Pharmacology, Harvard Med. Sch., Boston, MA 02115.

Immunocytochemical studies have demonstrated the presence of vasoactive intestinal peptide (VIP)-like immunoreactivity in adrenal medullary neurons. Although the function of VIP in the adrenal medulla is not known, the finding that VIP increases DOPA synthesis in PC-12 cells and rat chromaffin cells in culture (Tischler et. al., Neurosci. Lett. 61:141, 1985) suggests that it may play a role in the regulation of catecholamine biosynthesis. In the present study, we have examined the effects of VIP on catecholamine synthesis in suspension cultures of bovine adrenal chromaffin cells and characterized the VIP-induced activation and phosphorylation of tyrosine hydroxylase (TH).

Catecholamine biosynthesis rates in intact chromaffin cells were estimated by $^{14}CO_2$ evolution following the decarboxylation of DOPA formed from ^{14}C-l-tyrosine. Addition of VIP to the cells accelerated synthesis in a dose-dependent manner. Maximal stimulation was 3-4 fold over basal levels and was achieved at 10 uM VIP. The actions of VIP were relatively specific since other peptides related to VIP (secretin, glucagon and PHI) had no effect on synthesis. To measure TH activation, cells were sonicated and gel filtered aliquots assayed for enzyme activity at pH 7.0 in the presence of subsaturating cofactor (0.10 mM 6-MPH$_4$). The results showed that TH activity was elevated 3-fold by prior treatment of the cells with 10 uM VIP. This activation was dose-dependent and occurred over the same concentration range as that observed for the effects of VIP on synthesis in intact cells. Enzyme activity reached maximal levels after one minute of VIP treatment and had declined only slightly in cells exposed to VIP for up to 16 minutes. In intact cells prelabeled with ^{32}Pi to label ATP stores, VIP increased the incorporation of ^{32}Pi into the 60 K subunit of TH. RP-HPLC analysis of tryptic fragments of ^{32}P-TH from VIP-treated cells demonstrated an increase in the ^{32}P content of a single phosphopeptide (Soc. Neurosci Abstr., 1987). Since this is the same peptide phosphorylated by cAMP analogs and forskolin, additional experiments were carried out to determine whether VIP affected cAMP accumulation in the cells. The cAMP content increased significantly (2-fold) following exposure of the cells to 10 uM VIP.

Our findings support the hypothesis that VIP plays a role in the regulation of catecholamine synthesis in the adrenal medulla and that cAMP is involved in the mechanism of action of VIP. Supported by USPH NS11061 and NS12651, MH00162.

457.14 RAPID IN SITU ACTIVATION AND MULTIPLE SITE PHOSPHORYLATION OF TYROSINE HYDROXYLASE BY ACETYLCHOLINE IN BOVINE ADRENAL CHROMAFFIN CELLS: RELATIONSHIP BETWEEN SITES PHOSPHORYLATED AND ENZYME ACTIVATION. J.C. Waymire, J.P. Johnston, K. Lickteig* and G. L. Craviso. Dept. of Neurobiology and Anatomy, Univ. Texas Med. Sch., Houston, TX 77225.

Considerable evidence supports the concept that in situ activation of tyrosine hydroxylase (TH) is associated with phosphorylation of the enzyme on one or more sites. Here we report the use of RP-HPLC to characterize the number of sites on TH phosphorylated in situ in isolated chromaffin cells and the relationship between the sites phosphorylated and the activation of TH in response to acetylcholine (ACh).

Tryptic digests of the 60 K subunit of TH, phosphorylated in situ by incubation of the cells with ^{32}Pi for 90 min, yielded 7 phosphopeptides when subjected to RP-HPLC using a 0-17% acetonitrile gradient in 0.1% TFA (peptides were numbered 1 through 7 according to their order of elution). An identical chromatographic profile was obtained for tryptic fragments of immunoprecipitated ^{32}P-TH, indicating that all 7 phosphopeptides of the 60 K band were derived from TH. Because rechromatography of each peptide in a second RP-HPLC solvent system yielded single radioactive peaks, each peak appeared to comprise only one labeled peptide. When ^{32}P-labeled cells were incubated with 100 uM ACh, the phosphate content of 5 phosphopeptides (#3-7) was increased. Time course studies demonstrated 2 phosphorylation patterns in response to ACh. A rapid 5-fold increase in the ^{32}P content of peptides 3,5 and 6 occurred which became maximal in 10-15 sec and returned to basal conditions by 2-3 min, even if ACh was still present in the incubation medium. A slower increased ^{32}P incorporation occurred in peptides 4 and 7 which reached a plateau in 4-5 min and gradually returned to basal conditions by 16 min. The addition of EGTA 10 min prior to ACh blocked the enhanced phosphorylation of all 5 peptides, indicating that the in situ phosphorylation of TH by ACh is calcium-dependent. To determine the relationship between the multiple site phosphorylation of TH and enzyme activation, cells were treated with 100 uM ACh and TH activity measured in vitro after sonication of the cells. The enzyme was maximally activated by 15-30 sec after the addition of ACh but activity declined rapidly, approaching basal levels by 4 min. A comparison of the time course for TH activation vs phosphorylation indicated that activation closely paralleled the amount of ^{32}P incorporated into peptides 3, 5 and 6, whereas there was an inverse relationship between TH activation and enhanced ^{32}P incorporation into peptides 4 and 7. Supported by USPH NS 11061.

SEROTONIN AND BIOGENIC AMINES

458.1 A COMPARISON OF SEROTONIN, DOPAMINE, AND NOREPINEPHRINE LEVELS IN SEVERAL SPINAL CORD REGIONS IN THE FETAL, JUVENILE, AND ADULT GUINEA PIG (Cavia percellus). L.C. Abbott, B. Weber, and R.M. Bowker. Dept. of VCAPP, Washington State University, Pullman, Washington 99164-6520.

It has long been known that changes occur in concentrations of neurotransmitters in the developing central nervous system (CNS). Growth of biogenic amine-containing neuronal systems within the spinal cord and subsequent changes in location and amounts of these neurotransmitters are postulated to play a role in the maturation of function in both descending and ascending spinal cord systems. An important question addressed by this work is the relationships of changing levels of biogenic amines in different regions of the spinal cord in the developing guinea pig. Pregnant dams were anesthetized, and the fetuses were removed via cesarean section at three different timepoints during the last third of gestation and assessed for developmental age. Either the fetuses were immediately perfused for immunocytochemistry or specific CNS tissues were removed and processed for high pressure liquid chromatography-electrochemical detection (HPLC-EC). Perfusion started with warm, 0.9% saline, then 3.5-4% paraformaldehyde in phosphate buffer followed by sectioning (22-30 μm) and incubation of sections for 24-36 hours in antiserum raised to serotonin (5HT) or dopamine (DA). The sections were processed for routine immunocytochemistry; controls were also done. Processing for HPLC-EC consisted of homogenizing tissues in HPLC mobile phase and measuring norepinephrine (NE), 5HT, and DA concentration in the supernatant relative to the amount of protein present (measured by Lowry). The three biogenic amines were compared in cervical, thoracic, lumbar, and sacral spinal cord regions from the different-aged fetuses as well as juvenile and adult guinea pigs. 5HT and NE concentration tended to increase with fetal age, while DA either decreased or remained unchanged. The three biogenic amines had different timepoints of maximum concentrations in different levels of the spinal cord. These quantitative results suggest that these neurotransmitters are important in establishing functional relationships in the developing spinal cord. Supported by NIH grants RR00515 and NS22321, and NS24388.

458.2 COEXISTENCE OF SEROTONIN AND SUBSTANCE P WITHIN CELL BODIES IN THE RAT BRAINSTEM. S. Tallaksen Greene, M.W. Wessendorf, and R. Elde. Dept. of Cell Biology and Neuroanatomy, Univ. of Minnesota, Minneapolis, MN 55455

We have previously reported the distribution in the brain of fibers immunoreactive for both serotonin (5HT) and substance P (SP). The cell bodies of origin of these fibers have not been determined. However, coexistence of 5HT and SP immunoreactivity (5HT/SP-ir) has been reported within cell bodies in medullary raphe nuclei and the ventral reticular formation. The purpose of this study was to provide a detailed map of the distribution of cells in which 5HT and SP appear to coexist and to determine the percentage serotonergic cells immunoreactive for substance P in discrete brainstem regions.

Adult Sprague-Dawley rats treated with colchicine (50-75 μg in 10 μl into the lateral ventricle or cisterna magna) were perfused through the ascending aorta with Zamboni's fixative. Brains were removed and 10μ tissue sections were cut on a cryostat. Tissue sections were stained using a two-color immunofluorescence procedure for the simultaneous visualization of 5HT and SP immunoreactivities. 5HT immunoreactive perikarya were counted and classified according to location and the presence or absence of coexistent SP-ir. The data were expressed as the number of 5HT/SP-ir cells per 10μ section, and percentage of serotonergic cells containing SP-ir using the following equation:

$$\frac{\text{number of cells stained for 5HT + SP}}{\text{number of cells stained for 5HT}} \times 100$$

Consistent with previous studies, we observed an extensive distribution of 5HT cell bodies extending throughout the brainstem from the caudal medulla to the rostral mesencephalon. 5HT/SP-ir cells were identified within the caudal medial brainstem corresponding to the B1, B2, and B3 cell groups of Dahlstrom and Fuxe. Regional differences were observed with respect to both the total number of 5HT/SP-ir perikarya and the percentage of serotonergic cells containing substance P. In the B1 cell group the majority of 5HT/SP-ir cells was observed in nucleus raphe pallidus. Double labeled cells appeared to constitute virtually all of the serotonergic neurons in the caudal portion of this nucleus. Cells labeled for 5HT but not SP were most common in the rostral pole of raphe pallidus and in the region ventral to the lateral reticular nucleus. B2 contained a smaller number of 5HT/SP-ir neurons than B1. However, more than 80% of the serotonergic cells in nucleus raphe obscurus were observed to contain SP. In B3, the majority of 5HT/SP-ir cells was observed within the caudal portion of the nucleus raphe magnus where more than 90% of the serotonergic neurons were immunoreactive for SP. The percentage of double labeled cells within raphe magnus decreased rostrally to less than 5%. No SP immunoreactivity was observed within serotonergic cell bodies in the B4 -B9 cell groups.

These results indicate that 5HT/SP-ir cells make up an anatomically discrete subpopulation of serotonergic neurons. Further studies are needed to determine the projection pathways and functional significance of these cells. Supported by NS 22665, DA 02148 and ImmunoNuclear Corp.

458.3 STIMULATION OF DORSAL RAPHE NUCLEUS (DRN) AND LOCUS COERULEUS (LC) INCREASES 5-HTP ACCUMULATION IN MEDIAL PREOPTIC NUCLEUS (MPN) BUT NOT IN MEDIAN EMINENCE (ME). S. L. Petersen*, R. D. Hartman* and C. A. Barraclough (SPON: L. Kapcala). Dept of Physiol, Sch. of Med., Univ. MD, Baltimore, MD 21201.

Both the hypothalamic noradrenergic and serotonergic systems have been implicated as regulators of LHRH neuronal activity. While considerable evidence exists to support the concept that increases in NE secretion evoke LH surges, the role of serotonin (5-HT) in this event has not been clearly defined. Recent studies showed that drugs which deplete 5-HT (e.g. PCPA) also abolish the diurnal increase in NE secretion which may be required for the surge release of LH. Moreover, the 5-HT and NE systems may be intimately linked such that when LC is electrically stimulated (ES), DRN neuronal activity (and thus 5-HT secretion) also increases. It is not known how projections from these midbrain 5-HT and NE systems interact within the the MPN to affect LHRH neurons.

We have begun a series of studies to examine whether ES of DRN or LC affects 5-HT secretion in hypothalamic regions which contain LHRH cell bodies (MPN) and terminals (ME). To evaluate changes in 5-HT secretion we measured the accumulation of 5-hydroxytrytophan (5-HTP) following blockade of l-aromatic amino acid decarboxylase with NSD-1015 (NSD). Estrogen-treated rats received NSD in saline (100 mg/kg) i.p. and were sacrificed at various times after injection. Untreated rats, sacrificed at 0 time before NSD, provided the initial 5-HTP control values. All brains were frozen-sectioned and micropunches of ME and MPN were obtained and analyzed for catechol- and indoleamine concentrations using HPLC with ESA Coulochem detection. To determine the rate of 5-HTP accumulation, rats were sacrificed at 0, 10, 20, 30 and 40 min after NSD treatment. A linear accumulation of 5-HTP occurred over 30 minutes and 5 HIAA, DA, E and DOPAC declined linearly over the same time period. 5-HT concentrations decreased during the first 10 min but, thereafter, they remained unchanged. To determine whether DRN axons project to the MPN and ME, we ES DRN (100 uA, 10 Hz, 1 msec duration, 15 sec on/off) for 30 minutes after NSD administration (STIM-DRN). None of the control rats received ES. MPN 5-HTP levels increased significantly in STIM vs SHAM rats, but surprisingly, no differences occurred in ME 5-HTP levels. We also determined that following bilateral LC-ES, 5-HTP accumulated in MPN in a manner similar to that observed after DRN-ES but again, no changes occurred in ME 5-HTP levels.

These data suggest that: (1) 5-HT axons from DRN project to MPN but not to ME; (2) LC-ES activates both NE and 5-HT neurons whose axons reach LHRH containing regions in MPN. Thus, an increase in activity in one system (e.g. LC) could release both NE and 5-HT in MPN. How LHRH neurons respond to this dual signal input remains to be resolved. Supported by Research Grand HD-02138.

458.4 IMMUNOCYTOCHEMICAL COLOCALIZATION OF 5-HYDROXYTRYPTAMINE AND TYROSINE HYDROXYLASE IN SYMPATHETIC NEURONS. [1]O. Häppölä, [1]S. Soinila, [2]T. Lahtinen*, [3]T.H. Joh and [4]H.W.M. Steinbusch. [1]Neurobiological Research Unit and [2]Department of Anatomy, University of Helsinki, Finland, [3]Department of Neurology, Cornell University Medical College, New York, USA, [4]Department of Pharmacology, Free University, Amsterdam, The Netherlands.

In our previous study (J. Auton. Nerv. Syst.,15:21, 1986) it was shown that a subpopulation of principal neurons (PN cells) of the rat superior cervical ganglion (SCG) exhibit 5-hydroxytryptamine immunoreactivity during early postnatal development. In this study, indirect immunofluorescence method was used to study expression of 5-hydroxytryptamine (5-HT) in principal sympathetic neurons of the adult rat SCG in different experimental conditions, in vivo. 5-HT immunoreactivity was also studied in organotypic cultures of the newborn rat SCG.

In the SCG of normal adult rats, 5-HT immunoreactivity was localized in small intensely fluorescent (SIF) cells but not in PN cells. In the SCG of rats, pretreated with nialamide, a monoamine oxidase inhibitor, and the 5-HT precursor, L-tryptophan, a large number of 5-HT-immunoreactive (5-HT-IR) PN and SIF cells were detected. The 5-HT-IR PN cells had long processes, and 5-HT-IR nerve fibers were observed traversing the ganglion. In SCG of rats, pretreated with colchicine, occasional 5-HT-IR PN cells were detected. Ligation of the main postganglionic nerve trunks of the SCG of normal rats, resulted in appearance of several 5-HT-IR PN cells and nerve fibers in the ganglion. Colocalization studies on the same section or on consecutive sections demonstrated that the 5-HT-IR PN cells were also immunoreactive for the catecholamine-synthesizing enzyme, tyrosine hydroxylase. 5-HT-IR neurons were also present in organotypic cultures of the SCG and the fiber outgrowth exhibited 5-HT immunoreactivity.

The results of the present study demonstrate that the superior cervical ganglion of the rat contains 5-HT-IR principal neurons, which are not detectable in normal animals but can be visualized 1) after ligation of the postganglionic nerve trunks, 2) after colchicine pretreatment or 3) after pretreatment with nialamide and L-tryptophan. The 5-HT-IR principal neurons also contain tyrosine hydroxylase immunoreactivity suggesting colocalization of 5-HT and catecholamines in some sympathetic neurons.

458.5 5-HYDROXYTRYPTAMINE-IMMUNOREACTIVE CELLS AND TRYPTOPHAN HYDROXYLASE ACTIVITY IN THE RAT SUPERIOR CERVICAL GANGLION. H. Paivarinta, D.H. Park, A.C. Towle and T.H. Joh. Dept. of Neurology, Lab. of Molec. Neurob., Cornell Univ. Med. Coll., 1300 York Ave, N.Y., NY 10021.

Sympathetic ganglia are composed of principal nerve (PN) cells and small intensely fluorescent (SIF) cells. Evidence has been presented to suggest that SIF cells may function as interneurons, endocrine cells or chemoreceptor cells. In addition to catecholamine, 5-hydroxytryptamine (5-HT) has been immunohistochemically detected in the rat superior cervical ganglion. During early postnatal development 5-HT immunoreactivity is observed both in PN cells and SIF cells. However, if adult rats are treated with nialamide and loaded with L-tryptophan, some large 5-HT-immunoreactive cells with typical appearance of PN cells is observed. Furthermore, glucocorticoid injections into early postnatal rats cause an increase in the number of SIF cells and induce the enzyme phenylethanolamine-N-methyltransferase, thus favoring adrenergic differentiation. In addition, the glucocorticoid treatment leads to a dramatic increase in the number of 5-HT-immunoreactive cells in the ganglia.

Currently it is not known whether 5-HT in the ganglia is synthesized locally or taken up from the blood stream or mast cells. In preliminary experiments the activity tryptophan hydroxylase, the rate limiting enzyme for 5-HT synthesis, was very low in the ganglia of 7-day-old or adult rats. The enzyme activity remained low in ganglia of 7-day-old rats injected for 5 days with hydrocortisone before sacrifice. Since this steroid treatment leads to a large increase in the number of 5-HT-immunoreactive cells, the unchanged enzyme activity in the ganglia may suggest a mechanism other than synthesis be responsible for that 5-HT in the sympathetic cells. To study the possibility that 5-HT is take up from the blood stream, we injected adult rats with the 5-HT uptake inhibitor fluxetine (10 mg/kg i.p.) 7 times every 12 hours. 5-HT-immunoreactive SIF cells were still seen in ganglia of fluoxetine-treated rats indicating that the 5-HT is likely to be synthesized in the SIF cells. Thus, the results are in some conflict with regard to the origin of 5-HT in sympathetic cells. Further studies are directed to determine the mechanism of origin of 5-HT in sympathetic cells.

458.6 SEROTONINERGIC INNERVATION OF MONKEY CINGULATE CORTEX. C.A. Kitt†, L.C. Walker†, M.E. Molliver§, T.L. Dellovade*† and D.L. Price†. †Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; §Dept. Cell Biology & Anatomy, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The primate neocortex receives a rich serotoninergic input from neurons located within the raphe complex. The present study used immunocytochemical methods and an antibody directed against serotonin to map the laminar distribution of serotoninergic fibers in the anterior and posterior segments of the cingulate cortex (Brodmann areas 23 and 24) of the rhesus monkey (Macaca mulatta). Cortical laminae were first identified in Nissl preparations: the anterior cingulate cortex is characterized by a thin layer III, the absence of layer-IV neurons, and densely stained, large cells in layers V and VI; and the posterior cingulate cortex has six distinct layers, with a clearly identifiable layer IV. In serotonin immunocytochemical preparations, at least two types of fibers were identified: fine-diameter axons with either small round or smooth, fusiform varicosities; and larger-diameter axons with large spherical varicosities. Both types of axons were seen in both anterior and posterior cingulate cortices. The anterior cingulate cortex shows a dense plexus of fibers in layers I and II; the majority of fibers in layer I course parallel to the pial surface, whereas those in layer II are primarily oriented perpendicular to the pial surface. Serotoninergic fibers are more sparse in layers III-VI. Serotoninergic processes appear to enter the anterior cingulate through the cingulum bundle, pass through layers V and VI, and terminate principally in superficial layers. In contrast, serotoninergic fibers appear to enter in the posterior cingulate cortex through layer VI, are densely packed and radially oriented towards the pial surface in layers III-V, and are more sparse in superficial layers than in anterior cingulate cortex. The results of this study suggest that serotoninergic neurons of the raphe project to specific cortical regions in distinctly different patterns. The anterior and posterior cingulate cortices may, therefore, be further differentiated by transmitter circuitry as well as by morphological and connectivity criteria.

458.7 IMMUNOHISTOCHEMICAL LOCALIZATION OF 5-HYDROXYTRYPTAMINE IN SMALL INTENSELY FLUORESCENT CELLS IN FROG SYMPATHETIC GANGLIA. Parviz Yavari* and Seppo Soinila (SPON: D. Campbell). Hatfield Marine Science Center, Oregon State University, Newport, OR 97365.

5-Hydroxytryptamine (5-HT) has been shown to play important modulatory roles in the amphibian sympathetic ganglia, including regulation of acetylcholine (ACh) release, control of ACh receptor sensitivity, augmentation of slow synaptic responses; as well as having direct hyperpolarizing action on neurons. However, it is not known if 5-HT exerts these actions in vivo. One way that 5-HT may affect the ganglia is via circulation. Alternatively, 5-HT may be localized in specific structures in the ganglia and may act locally. To our knowledge no report to date has described the localization of 5-HT in amphibian sympathetic ganglia. We have used immunohistochemical techniques to study localization of 5-HT in the pre- and postganglionic nerve trunks and 9 & 10th sympathetic ganglia of the bullfrog Rana catesbeiana. The tissues were immersion-fixed in 4% paraformaldehyde, cryostat and paraffin sections were cut and processed using both Coons' indirect immunofluorescence mehtod and Sternberger's peroxidase-antiperoxidase method. We detected no immunohistochemical reactivity for 5-HT in either pre- or postganglionic nerve trunks, nor in the principal ganglionic neurons. However, intense 5-HT immunoreactivity was seen in clusters of small intensely fluorescent (SIF) cells in ganglia 9 and 10. The localization of 5-HT to SIF cells in frog sympathetic ganglia opens up the interesting possibility that SIF cells may be involved in the above-mentioned modulation of ganglionic transmission by releasing 5-HT.

458.8 DISTRIBUTION OF MONOAMINERGIC NERVE TERMINALS IN THE OPTIC TECTUM OF GYMNOTID FISH. AN IMMUNOFLUORESCENCE AND IMMUNOCYTOCHEMICAL STUDY. E. Sas and L. Maler, Dept. of Anatomy, Faculty of Health Sciences, Ottawa, Canada, K1H 8M5.

Our previous studies investigated the diverse cell types of the optic tectum of gymnotid fish, the sources of tectal afferents, their laminar segregation, and the stratified distribution of acetylcholinesterase containing axons in this structure.

The present work deals with the identification of monoaminergic terminals in the optic tectum of Apteronotus leptorhynchus with the aid of: I) Immunohistofluorescence techniques, and II) An immunocytochemical method for the differentiation of dopamine from norepinephrine containing fibers. The protocol of Smeets was followed, using antiserum raised against gluteraldehyde-conjugated dopamine, kindly provided by Dr. R.M. Buijs's laboratory. III) The immunocytochemical method of Steinbush et al 1978, was used for identification of serotonergic fibers.

In the optic tectum of Apteronotus, serotonin containing axons were present in the superficial sublamina of stratum opticum, at the stratum opticum/stratum fibrosum et griseum superficiale border, and dorsal part of mid stratum griseum centrale. In addition, some thicker fibers were observed ascending through stratum album centrale, whereas in the stratum periventriculare they formed small bundles which were obliquely cut, in transverse sections. Among the possible candidates for sources of serotonergic terminals in the tectum are the N. raphe dorsalis, and the mesencephalic dorsal tegmental nucleus. These nuclei project to the tectum and part of their neuronal population showed strong immunoreactivity for serotonin.

Dopaminergic fibers were distributed as follows: a) in a bilayered fashion in stratum opticum, b) as a loose plexus at the deep stratum fibrosum et griseum superficiale/stratum griseum centrale border, c) as ascending fibers in stratum griseum centrale, and d) forming a meshwork in stratum periventriculare. The dopaminergic fibers appeared to be confined mainly to the known retinorecipient layers. Although there is no report in the literature of the existence of dopaminergic fibers in the optic tectum of fish; recent studies (Hökfelt et al 1984) have shown a subpopulation of dopamine containing ganglion cells in the retina, thus supporting our findings of possible dopaminergic retinotectal projections in Apteronotus.

An attempt was made to correlate the laminar distribution of these monoaminergic fibers, with some of the known sources of tectal afferents, terminating in these layers. The general organization and morphological appearance of these monoaminergic plexuses in the optic tectum of Apteronotus, will be compared with reports in other fish; and the possible relevance of these neurotransmitters in the modulation of sensory-motor processing will be discussed.

458.9 [^{3}H]TRYPTAMINE BINDING SITES IN BRAIN: LACK OF ASSOCIATION WITH MONOAMINE OXIDASE ENZYMES. A.M. Martino-Barrows[1], D.C. Perry[2], L. Grimm[2], M.L. Jones[1], and K.J. Kellar[1]. Departments of Pharmacology, Georgetown University School of Medicine[1] and George Washington University School of Medicine[2], Washington, DC.

[^{3}H]Tryptamine labels high affinity binding sites in rat brain with a unique pharmacological profile (Cascio and Kellar, Eur. J. Pharmacol. 95: 31, 1983). In vitro autoradiographic techniques demonstrated a distinct heterogeneous distribution in rat brain, and it also revealed the presence of a second class of tryptamine sites ("T-2") in choroid plexus (Perry, J. Pharmacol. Exp. Ther. 236: 548, 1986). It remains unclear, however, whether [^{3}H]-tryptamine is binding to a true receptor or perhaps to membrane-bound monoamine oxidase (MAO) enzyme. In homogenate binding experiments, it was found that the presence of pargyline (a MAO inhibitor) is required for specific binding. Optimal binding occurs with 10 uM pargyline; higher concentrations decrease binding, with an IC_{50} of approximately 400 uM. This suggests that lower concentrations of pargyline protect the radioligand from enzymatic destruction, while only at higher concentrations does pargyline begin to compete at the tryptamine binding site. Comparisons with pargyline, however, are complicated by the non-competitive nature of its interaction with MAO. A better comparison is with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), which like tryptamine is an MAO substrate. [^{3}H]MPTP has been shown to label membrane-bound MAO in rat brain (Rainbow et al., Brain Res. 330: 337, 1985). In homogenate and quantitative autoradiography experiments, tryptamine inhibits [^{3}H]tryptamine binding at 2-3 nM potency, whereas the IC_{50} of tryptamine vs. [^{3}H]MPTP is >1000 nM. Similarly, while [^{3}H]MPTP has a K_d of approximately 20 nM, the IC_{50} of MPTP vs. [^{3}H]tryptamine is >1000 nM. Finally, the autoradiographic distribution of [^{3}H]-tryptamine, [^{3}H]MPTP and [^{3}H]pargyline in rat brain were compared. Although similarities were seen, a number of significant differences were readily apparent. While we cannot rule out the possibility that a component of [^{3}H]tryptamine binding is to membrane-bound MAO, the present data argue strongly for an independent identity for tryptamine binding sites.

458.10 **CELLULAR DISTRIBUTION OF MONOAMINE OXIDASE A IN INTESTINAL EPITHELIUM IS CONSISTENT WITH ITS HYPOTHESIZED PROTECTIVE ROLE AGAINST VASOACTIVE DIETARY AMINES**
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Patients treated with inhibitors of monoamine oxidase (MAO) A sometimes experience life-threatening hypertensive episodes after ingestion of foods containing dietary tyramine (the so-called "cheese effect"). For this reason, it has been proposed that intestinal MAO A provides an effective metabolic barrier to vasoactive amines in the diet. To investigate this hypothesis, we have localized MAO A in histological sections of human ileum using a newly-isolated monoclonal antibody to human placental MAO A (MAO A-4D3), which in contrast to previously-reported monoclonal antibodies to human MAO A (Kochersperger et al. J. Neurosci. 5:2874, 1985), was made using the non-secreting myeloma parent P3/X63.653. Like the four previously-described antibodies, MAO A-4D3 immunoprecipitates Triton X-100-solubilized MAO A with little inhibition of catalytic activity. However, at equivalent dilutions of ascites fluid, MAO A-4D3 stains MAO A in immunoblots of the purified enzyme or Triton X-100 extracts of placental mitochondria more intensely than any of the other antibodies. Competitive binding experiments indicate that MAO A-4D3 and two previously isolated MAO A-specific antibodies (MAO A-7B10 and MAO A-3C9, from two different BALB/c mice) recognize overlapping or identical binding sites on MAO A.

Immunocytochemical staining with MAO A-4D3 of histological sections of normal human ileal tissue reveals marked staining only in the epithelial layer of the villi (enterocytes), consistent with the proposed physiological role of MAO A as a metabolic barrier in the intestine. In contrast, immunocytochemical staining with the MAO B-specific antibody, MAO B-1C2, revealed little staining. The presence of MAO A in enterocytes, which constitute a single-cell layer separating the inside from the outside of the body, is strikingly reminiscent of the localization of MAO A in the syncytiotrophoblast of placental villi (Thorpe et al. J. Histochem. Cytochem. 35:23, 1987) at the boundary between mother and fetus. These results suggest that additional epithelial cells and tissues should be examined to determine if MAO A expression is a common characteristic of epithelial "barrier" tissues. [Supported by NS 19543]

458.11 PHOTOINACTIVATION OF MONOAMINE OXIDASE A (MAO-A) AND B IN HUMAN BRAIN. M-C. Hsu*, K. J-S Chen*, and J. C. Shih
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The effects of 4-fluoro-3-nitrophenyl azide (FNPA) on monoamine oxidase (MAO) A and B in human Brain cortex were studied. In the dark, FNPA competitively inhibited both MAO-A (Ki=4.5 μM) and MAO-B (Ki=2.0 μM) with similar affinities. Upon irradiation of increasing concentration of FNPA with the brain homogenate, the Vmax was decreased whereas the Ki was not changed for both types of MAO. This result suggests that a covalent linkage was formed between the enzyme and the photolyzed FNPA, and that the FNPA binding site may be the same as the substrate. Additional evidence for the photoinduced covalent binding of FNPA to both MAO-A and MAO-B was that there was no recovery of activity upon extensive washing of the photolyzed FNPA-enzyme complex. Interestingly, the photodependent inhibitory effect of FNPA on MAO-B was much more effective than MAO-A. A 50% photodependent inhibition of MAO-B activity was obtained with 1-2 μM FNPA, whereas 15-30 μM was required for MAO-A. These results suggest that there is a conformational or a structural difference in the active sites of the two types of MAO, and FNPA may be useful for probing this region of the active site. (Supported by NIMH Grant No. MH 37020 and MH 39085)

458.12 A COMPARISON OF CHRONIC PHENELZINE AND DEUTERATED PHENELZINE ON RAT BRAIN MONOAMINE LEVELS. L.E. Dyck*, A.V. Juorio*, D.A. Durden* and A.A. Boulton. Psychiatric Research Div., Saskatchewan Health, CMR Bldg., Univ. of Saskatchewan, Saskatoon, Sask., S7N 0W0, Canada.

Previous studies have shown that the antidepressant, phenelzine (PLZ) is more potent behaviourally and biochemically when its alkyl side chain is deuterated. The increased behavioral efficacy of acute administration of deuterated PLZ ($[^2H_2]$-PLZ) appears to be correlated to increased levels of brain tryptamine (TRA) and β-phenylethylamine (PEA) rather than to other monoamines, such as serotonin (5-HT), dopamine (DA) or nordadrenaline (NA). A previous study comparing the effects of chronic administration of PLZ and $[^2H_2]$-PLZ showed that it increased DA, NA and 5-HT levels more than PLZ itself, but the differences between the drugs were rather small. We have extended this study by measuring the effects of chronic PLZ and $[^2H_2]$-PLZ on brain NA, DA, 5-HT, TRA, PEA, m-tyramine (mTA), p-tyramine (pTA) levels and rat brain MAO activity. Saline, PLZ, or α,α-$[^2H_2]$-PLZ (0.5 or 2.5 mg/kg) were administered s.c. for 13 days via mini-osmotic pumps. After this, the rats were killed and the striata dissected out. Monoamines, tyrosine, tryptophan, and metabolite levels were measured simultaneously using HPLC-ECD and mass spectrometric methods on aliquots of the homogenized striatal samples. Tyrosine and tryptophan levels were unaffected by PLZ or $[^2H_2]$-PLZ. The concentrations of DOPAC, HVA and HIAA were dose-dependently decreased by PLZ and $[^2H_2]$-PLZ; furthermore, $[^2H_2]$-PLZ decreased the amounts of these acids more than the equivalent dose of PLZ. DA levels were increased the same amount (130% of control) by all drug treatments; i.e. no dose or deuterium effects were observed. With the exception of PEA, qualitatively similar effects were found with all other amines measured; their amounts were increased dose-dependently and the effects of $[^2H_2]$-PLZ were greater than those of PLZ. While the levels of NA, 5-HT and 3-methoxytyramine were increased 8.3, 3.1 and 6.8 times greater than control by 2.5 mg/kg $[^2H_2]$-PLZ, the levels of TRA, mTA and pTA were increased even more (14.3, 12.8 and 28.1 times control respectively). In contrast, PEA levels were not increased by PLZ and only 2.5 mg/kg $[^2H_2]$-PLZ elevated PEA levels compared to saline. Rat cerebral MAO activity was inhibited dose-dependently by PLZ and by $[^2H_2]$-PLZ. Type A MAO was inhibited more than type B, and $[^2H_2]$-PLZ inhibited both types more than PLZ. The present study agrees with previous findings. In addition, it shows that the efficacy of PLZ was increased about 5-fold by deuteration, that $[^2H_2]$-PLZ increased TRA, mTA and pTA levels more than other monoamines, that PEA levels were least affected by the drug treatments, and that $[^2H_2]$-PLZ inhibited MAO more than PLZ.
(Supported by Saskatchewan Health and the MRC of Canada).

458.13 MULTIPLE ADENOSINE RECEPTORS IN THE RAT VAS DEFERENS PREPARATION. Susan J. Ward and Renee Cary, Department of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, New York, 12144.

It is generally recognized that adenosine (A) agonists inhibit the electrically stimulated release of norepinephrine in the rat vas deferens preparation. Studies evaluating potential CNS actions of A agonists (eg. analgesia, antipsychotic action) prompted the present study which was designed to characterize receptors located on nervous tissue in an isolated tissue preparation. Rat vasa deferentia preparations (RVD) were stimulated electrically at 0.1 Hz and bathed in warm oxygenated Krebs solution. A agonists and some antagonists inhibited contractions of the RVD; IC_{50} values are shown in Table 1. Reported selectivity for A_1 and A_2 receptors is shown in Table 1. The following compounds were evaluated for their effects on the inhibitory actions of adenosine agonists: the A antagonists 8-PT, DPX, and PAPCX, the phosphodiesterase (PD) inhibitor rolipram, and the mixed PD inhibitor antagonist theophylline.

Table 1

Agonist	IC50 (μM)	Selectivity	pA_2 vs 8-PT	pA_2 vs DPX	pA_2 vs PACPX
CHA	0.097	A1 >A2	7.04	7.39	7.92
CPA	0.112	A1 >A2	—	7.11	7.97
NECA	0.143	A1=A2	6.67	7.29	—
(-) PIA	0.291	A1 >A2	7.13	7.17	7.28
2-CADO	0.479	A1=A2	see text	see text	6.49
(+) PIA	2.02	A1 >A2	6.83	7.11	7.94

Theophylline, 30 and 100 μM produced a small, non-competitive rightward shift in the concentration-effect (CE) curves for each of the agonists, but at 300 μM, either had no effect, or potentiated the A agonists. These potentiating actions were mimicked by rolipram (0.3 μM). Both theophylline and rolipram inhibited twitch height in the RVD, whereas the non-PD inhibitors 8-PT, CPX and PAPCX did not potentiate A agonists or inhibit twitch height. pA_2 values for 0.3 - 30 μM 8-PT to antagonize the A1 selective agonists CHA, CPA, (-) PIA (+) PIA and the mixed A1/A2 agonist NECA ranged between 6.8 and 7.1; pA2 values for 0.1-3 DPX to antagonize the same agonists ranged from 7.1 to 7.4 (Table 1). For both antagonists, the slopes of the schild plots for the antagonism of the mixed A1/A2 agonist 2-CADO were less than 1. The A1 selective antagonist PAPCX (0.03 - 0.3 μM) antagonized CPA, (-) PIA, (+) PIA and CHA with pA2 values ranging from 7.3 to 8.0. These concentrations of PAPCX produced less than a 3-fold shift in the CE curves for 2-CADO and NECA. Higher concentrations of PACPX did antagonize 2-CADO and NECA, the pA2 value for CADO-PACPX being 6.5. The data caution against the use of A antagonists that are also PD inhibitors as tools in these types of assays. The data also suggest existence of 2 populations of receptors in the RVD that are consistent with an A1/A2 classification.

458.14 SELECTIVE LOCALIZATION OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS IN MOUSE BRAIN. L. Lima*, R. Walder*, F. Gil* and B. D. Drujan*. (SPON: H. Vanegas). Laboratorios de Neuroquímica y Virología, IVIC, Caracas, Venezuela.

Pixuna, a strain of intermediate virulence of venezuelan equine encephalomyelitis virus (VEEV), inoculated to 24 days old mice produces a time-dependent encephalitis. Minimal damage is associated with the subclinical stage. Prior to appearence of encephalitis there is a decrease in catecholamine level and turnover rate in some discrete brain areas, and a decrease in serotonin (5HT) turnover in raphe and cerebral cortex. Behavioural studies of infected animals have served to show that locomotor activity was affected in a heterogeneous manner and that there was a supersensitivity of 5HT receptors. A selective affinity of different viruses for distinct central nervous system areas has been reported. The aim of this work was to detect a tropism for brain areas that are known to present neurochemical changes during subclinical infection by VEEV. Pixuna strain was inoculated intracraneally with 300 and 1,200 LD_{50}, determined in suckling mice. Sacrifice was performed at 4 and 7 days post inoculation for the lower dose, and at 1, 2 and 3 days post-inoculation for the higher one. Raphe area, peri-raphe region, striatum, and cerebral cortex were dissected and fixed in a phosphate buffered glutaraldehide and paraformaldehide solution plus 30% sucrose for immunohistochemical examination, and with glutaraldehide in buffer cacodylate for electron microscopy. To locate viral antigens frozen sections (10 μm) were performed. Mouse immune ascitic fluid was used as the primary antiserum. Second antibody was anti-mouse IgG conjugated with fluorescin isothiocyanate. Signs of infection appeared around 7 days post-inoculation in mice receiving 300 LD_{50}, although viral antigens were spare in brain of sick animals. At the 4th day post-inoculation small amount of antigens was detected. A discrete localization was observed in raphe and mainly in regions circunding the raphe. Most of the immunoreaction was associated with mild vacuolation of the area. Immunoreactive material was rare in the cerebral cortex. Higher doses of the virus did not enhance immuno reactivity at the established time intervals after inoculation. Small amount of viral particles were seen in membranous cisternae of the peri-raphe area by electron microscopy. Viral tissue or cell tropism involves several interactions which might correspond to the early biochemical changes produced in infected animals.

459.1 SEROTONIN ALTERS THE I-V CURVE AND THE EFFECTS OF DEPOLARIZING PREPULSES IN THE CYCLIC AMP-DEPENDENT CURRENT IN NEURONS OF THE MOLLUSC Pleurobranchaea californica. R.-C. Huang & R. Gillette. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

In identified pedal ganglion neurons intracellular iontophoretic injection of cyclic AMP induced an inward current recorded under voltage clamp. Stepwise substitution of Na^+ by tetramethylammonium caused stepwise decrease of current amplitude, indicating a Na^+ dependent current. In contrast to the activation of the current by depolarization in other identified neurons of Pleurobranchaea (Gillette & Green, this meeting), the I-V relation of the cAMP-stimulated current in the pedal neuron was approximately flat from -70 to -40 mV and rapidly decreased at more positive voltages. Conditioning pulses of depolarization and spiking caused transient partial suppression of cAMP response amplitude, which recovered over several minutes.

Bath application of 10 µM serotonin invariably induced a large and sustained inward current. Concomitantly, the I-V relation of the cAMP-stimulated Na^+ current was altered: response amplitudes at holding voltages greater than -30 mV were reduced in both amplitude and duration. Changes in the curve relating cAMP injection current to the Na^+ current response suggest serotonin acts in part by increasing endogenous levels of cAMP.

Serotonin also acted to reverse the suppressive effect of conditioning depolarization. In the presence of serotonin, the cAMP responses were either unaffected by depolarizing prepulses or, more often, were potentiated. These data show that serotonin alters the responsiveness of these neurons to cAMP as well as the action of membrane potential on the cAMP-dependent ion channels.

459.2 INOSITOL 1,4,5-TRISPHOSPHATE MIMICS THE HYPERPOLARIZATION INDUCED BY 5-HT IN CAT SPINAL MOTONEURONS IN VIVO. L. Zhang and K. Krnjevic, Departments of Anaesthesia Research and Physiology, McGill University, Montreal, Que. Canada, H3G 1Y6

Motor nuclei in vertebrate lumbosacral cord receive a 5-HT-ergic descending innervation from brain stem; however the actions of 5-HT on spinal motoneurons remain controversial. There are indications that in rat cerebral cortex, 5-HT stimulates inositol phospholipid breakdown, and that the effect of 5-HT is mediated by G protein in hippocampal pyramidal cells. We examined 5-HT's action and its intracellular mediation in cat lumbosacral motoneurons by simultaneous intracellular recording and intra- or extracellular iontophoresis.

Cats were anesthetized with pentobarbitone and immobilized with succinylcholine chloride (i.v. perfusion). Released by a positive current of 40-160 nA for 1-3 min, 5-HT hyperpolarized the membrane potential by 3-21 mV, increased the spike height and its maximal rate of rise (n=16), but without changing the input resistance, spike duration, afterhyperpolarization as well as postsynaptic potentials. The effects of 5-HT lasted as long as the recording (up to 60 min) and they could not be reversed by intracellular injection of Cl^-. Similar effefcts were induced by raising the arterial blood pressure (15-50 mm Hg) by injecting adrenalin or increasing i.v. perfusion rate (n=6).

To mimic the intracellular mediation of 5-HT action, the non-hydrolysable GTP analogue $GTP_{\gamma S}$ and GDP analogue $GDP_{\beta S}$ were injected into motoneurons. $GTP_{\gamma S}$ did not mimic the action of 5-HT (n=12), while it depolarized membrane potential. In the presence of $GDP_{\beta S}$, 5-HT still caused a hyperpolarization and increase in spike height, suggesting that G protein may not be involved in 5-HT action. In contrast, intracellular injection of inositol 1,4,5-trisphosphate (IP3) (2-5 nA, 0.5-1 min) mimicked the 5-HT action, by inducing a long-lasting hyperpolarization (up to 30 min) and increasing the spike height and its maximal rate of rise, without change in input resistance (n=6).

Present data suggest that the hyperpolarization without change in input resistance, may be due to facilitation of electrogenic Na-K ATPase activity by 5-HT, and that IP3, as intraellular messenger, mediates the action of 5-HT receptor. Since IP3 stimulates the release of internal Ca2+, internal Ca2+ may modulate the activity of Na-K ATPase.

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459.3 SEROTONIN REUPTAKE BLOCKERS INHIBIT THE FIRING RATE OF SEROTONERGIC NEURONS OF THE DORSAL RAPHE IN VITRO. G.C. Rigdon and C.M. Wang. Dept. of Pharmacology, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

Stimulation of 5-HT1a receptors results in inhibition of firing rate of 5-HT neurons of the dorsal raphe. The in vitro brain slice preparation provides a means of obtaining IC50 values for compounds which inhibit neuronal firing. This provides a physiological measure which can be used to determine concentration-response curves for comparison with neurochemical/receptor binding data. Concentration response curves for many 5-HT1a agonists have been determined using in vitro electrophysiological techniques. The object of this experiment was to determine if this in vitro procedure could be used to screen for compounds which act by blocking transmitter reuptake, thereby indirectly stimulating 5-HT1a autoreceptors. We investigated the effects of imipramine and fluoxetine, two antidepressent drugs shown to block 5-HT reuptake, on the firing rate of 5-HT dorsal raphe neurons in vitro. Extracellular single unit recordings were obtained from superfused, 300 micron slices of rat midbrain. Because few 5-HT neurons in the dorsal raphe are spontaneously active in vitro, phenylephrine (10 µM) was added to the superfusate to maintain a basal firing rate. 5-HT dorsal raphe units were identified by their slow (0.25-5.0/sec), steady firing rate, and by an inhibitory response to 8-OHDPAT or 5-HT application. The effects of imipramine administered alone in the superfusate and imipramine potentiation of a minimally effective concentration of 5-HT (0.1 µM, mean 10.9% inhibition) were studied. Imipramine (concentration range = 0.5-25 µM) administered alone inhibited the firing rate of 5-HT neurons in a dose dependent manner (IC50 = 5.8 µM). The IC50 for imipramine enhancement of the effects of 0.1 µM 5-HT was 5.0 µM. The imipramine effects were blocked by 1-10 µM (-)propranolol, a 5-HT1a antagonist. Fluoxetine suppressed the firing rate with an estimated IC50 of 2-4 µM. The results demonstrate that rat 5-HT dorsal raphe neurons respond to administration of 5-HT uptake inhibitors in the same manner in vitro as they do in vivo. Thus, in vitro single unit recording from the dorsal raphe provides a fast, simple means of obtaining a physiological measure of the potency of 5-HT reuptake blocking agents.

459.4 PRE- AND POST-SYNAPTIC ACTIONS OF 5-HYDROXYTRYPTAMINE IN THE RABBIT PARASYMPATHETIC NEURONS. T. Tokimasa, H. Hasuo*, T. Nishimura* and T. Akasu*. Department of Physiology, Kurume University School of Medicine, Kurume 830, Japan.

Intracellular recordings were made from neurons of vesical parasympathetic ganglia (VPG) isolated from the rabbit urinary bladder wall and maintained in vitro. 5-HT was applied either by superfusion or by pressure ejection from the tip of broken pipettes which contained 5-HT. 5-HT (0.01-100 µM) produced a dose-dependent membrane depolarization associated with an increase in membrane conductance, which reversed its polarity at about zero mV. The depolarization was not eliminated in a solution containing tetrodotoxin (TTX) or in a TTX-containing low calcium solution. These results suggested that 5-HT directly depolarizes the postsynaptic neurons. Removing all sodium ions from the perfusate markedly depressed the 5-HT induced depolarization. Removal of potassium ions slightly depressed the 5-HT response. Significant calcium or chloride dependence of the depolarization was not found. These results suggested that the 5-HT induced depolarization was due to an increased conductance for sodium and potassium ions. ICS 205-930 (0.01-100 nM) was found to be a potent antagonist of the 5-HT receptors. 100 pM ICS 205-930 shifted in parallel the dose-response curve of 5-HT to the right, implying a competitive antagonism. Methysergide (30 µM) was not effective antagonist. 8-OH-DPAT did not mimic the 5-HT response. Therefore, 5-HT_3 is most likely to the subtype mediating 5-HT action.

5-HT (0.5-10 µM) presynaptically suppressed the fast excitatory postsynaptic potential (epsp) in all cells tested. 8-OH-DPAT (1-10 µM) also depressed the amplitude of the fast epsp. The presynaptic action of 5-HT was not antagonized by ICS 205-930 (1-300 nM), methysergide (5-30 µM) or ketanserin (1-30 µM). It was less likely that receptor subtype endowed on the preganglionic terminals is 5-HT_2 or 5-HT_3.

459.5 IN VIVO AND IN VITRO ELECTROPHYSIOLOGICAL STUDIES INDICATE THAT BMY 7378 IS A PARTIAL AGONIST AT 5-HT_{1A} RECEPTORS IN THE RAT DORSAL RAPHE NUCLEUS. C.P. VanderMaelen, J.P. Braselton*, and G. Gehlbach. Preclinical CNS Research, Bristol-Myers Co., 5 Research Parkway, Wallingford, CT. 06492.

Numerous ligands which bind to 5-HT_{1A} receptors demonstrate agonist actions at dorsal raphe (DR) 5-HT autoreceptors, causing inhibition of firing of DR serotonergic neurons. Two β-adrenoceptor blockers, (-)-propranolol (Sprouse & Aghajanian, EJP., 1986, 128, 295-298), and pindolol (Gehlbach and VanderMaelen, Soc. Neurosci. Abst., 1987) block 5-HT_{1A} receptors as well, antagonizing the effects of 5-HT_{1A} agonists on DR 5-HT neurons. The compound BMY 7378 binds with high affinity to 5-HT_{1A} sites in the hippocampus (IC_{50}=2.4 nM; Yocca et al., EJP., submitted), and can antagonize the actions of the 5-HT_{1A} agonist 8-OH-DPAT in the DR, and also the hippocampus (Chaput & de Montigny, Soc. Neurosci. Abst., 1987). Present studies investigated further possible agonist and antagonist properties of BMY 7378 in the rat DR, using in vivo and in vitro electrophysiological preparations.

Standard techniques were used for extracellular single-unit recordings from rat DR 5-HT neurons. For in vivo studies, test drugs were administered i.v. or by microintophoresis to chloral hydrate anesthetized rats. For in vitro studies, rats were anesthetized with chloral hydrate, allowed to breathe 100% O_2 for at least 5 min., and then 400 μm thick Vibratome brain slices containing the DR nucleus were prepared. Superfusing artifical CSF contained, in mM: NaCl, 130; KCl, 5.0; $CaCl_2$, 1.25; $MgSO_4$, 1.25; $NaHCO_3$, 24; NaH_2PO_4, 1.25; d-glucose, 10.0. L-phenylephrine (PE; 10 or 30 μM) was present to provide tonic excitation of DR neurons.

For the in vivo studies, i.v. administration of BMY 7378 caused inhibition of firing of DR neurons (ED_{50}=0.03 mg/kg,), as did microiontophoretic administration. When iontophoretic administration of BMY 7378 was adjusted to produce only partial inhibition of firing, this usually resulted in antagonism of the inhibitory effects of iontophoretically applied ipsapirone (a 5-HT_{1A} ligand), and in some cases, of 5-HT as well. Similarly, in the brain slice experiments, BMY 7378 produced inhibition of firing of 5-HT DR neurons (IC_{50}=100-150 nM). To see if this inhibition was due to an agonist action at 5-HT_{1A} receptors (as opposed to α_1 block, for example), the 5-HT_{1A} antagonist pindolol was used. Pindolol (1 μM) blocked the inhibition of DR neuronal firing produced by BMY 7378. Finally, when conditions were adjusted so that BMY 7378 did not produce complete inhibition of firing (BMY 7378 = 200 nM; PE =30 μM), BMY 7378 antagonized the actions of the 5-HT_{1A} agonist gepirone ($p<0.001$). Therefore, because of the combination of agonist and antagonist actions displayed by this compound, it is concluded that BMY 7378 functions as a partial agonist at 5-HT_{1A} receptors in the rat DR nucleus.

459.6 PINDOLOL BLOCKS THE INHIBITORY EFFECT OF GEPIRONE, A 5-HT_{1A} AGONIST, ON THE FIRING OF SEROTONERGIC DORSAL RAPHE NEURONS IN THE RAT BRAIN SLICE. G. Gehlbach and C.P. VanderMaelen, Preclinical CNS Research. Bristol-Myers Company, Wallingford, CT. 06492.

The β-adrenoreceptor antagonist, pindolol, binds to 5-HT_{1A} receptors in the rat hippocampus (IC_{50} = 56.6 nM, D.K. Hyslop, personal communication). Propanolol, another β-adrenoreceptor antagonist with affinity for 5-HT_{1A} receptors, has been shown to block the suppressant effects of 5-HT_{1A} selective agonists on 5-HT containing neurons of the dorsal raphe (DR) nucleus of the rat (Sprouse and Aghajanian, Eur. J. Pharmacol., 1986, 128:295-298). Like other 5-HT_{1A} agonists, the antidepressant-anxiolytic candidate, gepirone, inhibits the firing of DR neurons (Gehlbach and VanderMaelen, Soc. Neurosci. Abst., 1985, 11:186). The present study examined the effect of pindolol on the inhibition of serotonergic DR neurons produced by gepirone in the rat brain slice.

Adult male albino Sprague-Dawley rats were anesthetized with chloral hydrate and allowed to breathe 100% O_2 for at least 5 minutes. Frontal sections 400 μm in thickness were cut through the DR nucleus and placed in a chamber through which 95% O_2, 5% CO_2 flowed. The slices were bathed in continuously flowing artificial CSF containing in mM: NaCl, 130; KCl, 5.0; $CaCl_2$, 1.25; $MgSO_4$, 1.25; $NaHCO_3$, 24; NaH_2PO_4, 1.25; d-glucose, 10.0. Phenylephrine (PE, 10 μm) was present to provide increased neuronal activity. Gepirone in concentrations of 100, 150 or 200 nM was added to the bath and its inhibitory effect on the firing of DR neurons was observed using extracellular single unit recording techniques. Both the percent inhibition, and the decrease in counts per 10 sec interval were measured. Recovery of firing rate to or close to the pre-drug firing rate was obtained for each cell, followed by a 15 min exposure to pindolol (1.0 μM). Then each cell was exposed to the combination of gepirone (at the initial concentration tested) plus pindolol (1.0 μM). Drug effects were always assessed 15 min after initiating exposure, when responses are essentially asymptotic. Control experiments also were carried out which showed no significant changes in the inhibitory effects of gepirone with repeated administrations.

Pindolol (1.0 μM) alone had virtually no effect on firing rates of DR neurons. However, pindolol significantly ($p<0.001$) antagonized the inhibitory effects of the 5-HT_{1A} agonist gepirone. The amount of inhibition produced by gepirone was reduced by 81% in the presence of 1.0 μM pindolol, when "% inhibition" was the dependent measure. It was reduced by 84% when "change in counts" was the dependent measure. In conclusion, the β-adrenoreceptor antagonist, pindolol, effectively blocks the actions of a 5-HT_{1A} agonist in the rat dorsal raphe nucleus and may serve as a useful tool in studying 5-HT_{1A} receptors and ligands.

459.7 **ELECTROPHYSIOLOGICAL EVIDENCE THAT SPIPERONE IS AN ANTAGONIST OF 5-HT_{1A} RECEPTORS IN THE DORSAL RAPHE NUCLEUS.** J.T. Lum and M.F. Piercey, CNS Research, The Upjohn Co., Kalamazoo, MI 49001.

Buspirone and 8-hydroxy-DPAT (DPAT), both of which bind to 5-HT_{1A} receptors, depress 5-HT neurons in the dorsal raphe (DR) where 5-HT_{1A} receptors exist. To confirm that this effect is mediated by 5-HT_{1A} receptors, one needs to show that it can be reversed by a 5-HT_{1A} antagonist. Spiperone binds to 5-HT_{1A}, 5-HT_2, and dopaminergic (DA) D_2 receptors. We have tested spiperone for its effects on DPAT and buspirone in the DR. Action potentials of single 5-HT neurons were identified by large positive-negative voltages and slow (1-2 spikes/sec), regular firing rates recorded from microelectrodes located in DR (Aghajanian et al., JPET 137:178, 1977), DPAT (ED50 = 1.7 ug/kg) and buspirone (ED50 = 15 ug/kg) dramatically depressed firing rates of 5-HT neurons. Spiperone, 1.0 mg/kg i.v., completely reversed the effects of 5-HT_{1A} agonists and occasionally increased rates above controls. Lower spiperone doses were weaker, and higher doses did not increase the effect and occasionally depressed 5-HT cells. Spiperone's actions were not due to antagonism of 5-HT_2 receptors since LY53837, a selective 5-HT_2 antagonist, was ineffective. Since spiperone is a D_2 antagonist, studies were undertaken to determine if DPAT or buspirone had effects on DA systems. Substantia nigra (SNPC) DA neurons were identified by large, long-duration action potentials with firing rates of 3-8 spikes/sec recorded from electrodes located in SNPC (Bunney et al., JPET 185:560, 1973). Doses of DPAT that silenced DR cells did not affect DA firing rates, nor did it reverse amphetamine depressions of DA neurons. Thus, there is little interaction between DA and 5-HT systems. Higher DPAT doses (1 mg/kg) reversed amphetamine-induced depressions. Buspirone, which antagonizes D_2 receptors, resembled a classic neuroleptic on DA neurons, with a potency for reversing amphetamine depressions (10 ug/kg) similar to that for depressing 5-HT cells (15 ug/kg). Thus, D_2 receptor antagonism does not block buspirone's depression of 5-HT cells. Since neither D_2 antagonism nor 5-HT_2 antagonism reverses the depression of 5-HT cells by these 5-HT_{1A} agonists, it is concluded that spiperone reverses this effect by its action as an antagonist of 5-HT_{1A} receptors.

459.8 EFFECTS OF ICI 169,369, A SELECTIVE 5HT_2 ANTAGONIST, IN ELECTROPHYSIOLOGICAL TESTS PREDICTIVE OF ANTIPSYCHOTIC ACTIVITY. J.M. Goldstein, L.C. Litwin, E.B. Sutton, and J.B. Malick. Department of Pharmacology, Stuart Pharmaceuticals, A Division of ICI Americas Inc., Wilmington, DE 19897.

Recent preliminary clinical studies have demonstrated that 5HT_2 antagonists (e.g., ritanserin, setoperone) have antipsychotic (AP) activity in man with a reduced incidence of extrapyramidal side effects (EPS) (Gelders et al., IVth World Cong Biol Psychiatr 1985, Abst 420.4; Ceulemans et al., Psychopharmacol 85,329,1985). Moreover, 5HT_2 antagonists have been shown to decrease haloperidol-induced dystonia and parkinsonism in the monkey (Korsgaard et al., Eur J Pharmacol 118,245,1985), further supporting these findings. These data suggest that in animal tests predictive of AP activity, 5HT_2 antagonists should be active.

In previous studies (Goldstein et al., Fed Proc 45,436,1986; Litwin et al., Pharmacologist 28,109,1986) we have shown that the 5HT_2 antagonists ritanserin and altanserin reverse the rate-suppressant effects of amphetamine (AMP) on A9/A10 DA neurons (a test predictive of AP activity). In addition, low doses of ritanserin were found to increase selectively the number of spontaneously firing A10 DA cells (Goldstein et al., Fed Proc 46,966, 1987), an effect which predicts the ability of an agent to induce depolarization inactivation (DI) after chronic treatment (typical AP's cause DI of both A9/A10 DA cells whereas atypical agents with reduced EPS liability only cause DI of A10 DA cells).

We now report on the effects of ICI 169,369 ((2-(dimethylaminoethylthio)-3-phenylquinoline hydrochloride), a selective and potent 5HT_2 antagonist (Blackburn et al., Br J Pharmacol 90, 256P &277P, 1987), in electrophysiological tests predictive of both AP activity (reversal of AMP on A9/A10 DA cells) and EPS liability (increase in spontaneously active A9 DA cells). ICI 169,369 produced a dose-related reversal of AMP-induced suppression of A9 (ED_{50} = 1.5 mg/kg, i.v.) and A10 (ED_{50} = 0.9 mg/kg, i.v.) DA cell firing. On the basis of the lower ED_{50} in the A10 vs. the A9 area, ICI 169,369 may exhibit greater selectivity on DA cells in the A10 area. This possibility was explored by determining the effects of oral treatment of ICI 169,369 on the number of spontaneously active A9/A10 DA cells. ICI 169,369 (10 mg/kg, p.o.) produced a selective increase in the number of active cells in the A10 area, whereas at higher doses (20-40 mg/kg, p.o.), this selectivity was lost. On the basis of these acute data, ICI 169,369 may produce DI of A10 cells (AP activity) at a dose that does not produce DI of A9 cells (lack of EPS liability). Chronic studies are underway to confirm the predictability of the acute data. The overall results with ICI 169,369 suggest that this agent should demonstrate AP activity in man with a reduced likelihood for EPS. ICI 169,369 is currently undergoing clinical trials.

459.9 ELECTROPHYSIOLOGICAL RESPONSES OF HIPPOCAMPAL PYRAMIDAL CELLS TO SEROTONIN 5-HT-1A AND 5-HT-1B SELECTIVE AGONISTS: A COMPARATIVE STUDY WITH DORSAL RAPHE NEURONS. J.S. Sprouse and G.K. Aghajanian, Dept. of Psychiatry, Yale University Sch. of Med., New Haven, CT 06508.

The recently described 5-HT-1 binding site subtypes (5-HT-1A, 5-HT-1B, 5-HT-1C) are characterized by a marked regional distribution of binding densities in rat brain (Pazos & Palacios, Brain Res. 346: 205, 1985). In particular, 5-HT-1A sites are concentrated in the CA1 hippocampal pyramidal cell layer and in the dorsal raphe nucleus. Consistent with these binding data, selective 5-HT-1A agonists closely mimic the inhibitory effects of 5-HT on dorsal raphe cell firing whereas 5-HT-1B agonists are comparatively weakly active. Furthermore, in intracellular recordings, the maximal hyperpolarizing effect of 5-HT-1A agonists slightly but consistently exceeds that of 5-HT itself (Sprouse & Aghajanian, Synapse 1: 3, 1987). A systematic examination of the effects of 5-HT-1A and 5-HT-1B selective agonists on spontaneously firing CA1 hippocampal pyramidal cells has not been reported previously.

Extracellular recordings from CA1 pyramidal cells were made from unanesthetized rats transected to produce a low cerveau isole. Compounds were applied by microiontophoresis and included three 5-HT-1A agonists, 8-OH-DPAT, ipsapirone (IPSA; TVX Q 7821) and LY 165163 (PAPP), and two 5-HT-1B agonists, mCPP and TFMPP, as well as 5-HT.

Microiontophoretic application of 5-HT reversibly suppressed CA1 pyramidal cell firing within the same current-dose range as that observed in the dorsal raphe. In contrast to the dorsal raphe, however, neither the 5-HT-1A or 5-HT-1B agonists displayed potencies comparable to 5-HT when directly applied to the same cells: IPSA, PAPP, mCPP and TFMPP only weakly slowed spontaneous firing; 8-OH-DPAT more closely mimicked 5-HT but only at currents which substantially exceeded those required to supress dorsal raphe neurons. Co-iontophoreses of 5-HT-1A and 5-HT-1B agonists were no more effective than individual applications.

In summary, the inability of CA1 pyramidal cells to distinguish 5-HT-1A and 5-HT-1B agonists is in sharp contrast to the striking differences observed for these compounds with dorsal raphe neurons. Consistent with these findings is the notion that 5-HT-1A compounds are full agonists on dorsal raphe neurons but at best only partial agonists on pyramidal cells. Alternatively, it is possible that the large suppressant effects of 5-HT on pyramidal cells may involve actions at sites in addition to those at 5-HT-1A receptors.

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459.10 EFFECTS OF IONTOPHORETICALLY APPLIED 5-HT AGONISTS ON GRANULE CELL ACTIVITY IN THE RAT CEREBELLUM

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Serotonin immunoreactive fibers from the nucleus reticularis gigantocellularis, then paragigantocellularis and the n. pontis oralis have been found within the cerebellar granule cell layer of the rat; however, the physiological characterization of this serotonergic input has not been defined. The present study was designed to determine the effects of iontophoretically applied serotonin (5-HT) and 5-HT_{1b} putative agonist trifluromethylphenylpiperazine (TFMPP) on granule cell spontaneous activity in urethane anesthesized rats. A single barrel extracellular recording electrode was attached to the multibarrel electrode and filled with 2 M NaCl saturated with fast green. The iontophoretic barrels contained 0.15 M NaCl for continuous current balancing, 1.0 M mono-sodium glutamate, 0.4 M 5-HT creatine sulfate, 0.2 M TFMPP or 0.01 M methysergide maleate, a serotonin receptor antagonist.

Of the 108 granule cells tested with 5-HT, 39% exhibited significant decreases in firing frequency, 20% exhibited significant increases in firing frequency and 41% were not effected. Of the 29 granule cells tested with TFMPP, 72% exhibited significant decreases in spontaneous activity and 28% were not effected. Concurrent application of 0.01 M methysergide did not block the 5-HT or the TFMPP induced suppressive effects. Serotonin receptors have been primarily classified into 3 types; 5-HT_1, which is associated with decreases in neuronal activity, 5-HT_2 associated with increases in activity, and a third presynaptic autoreceptor type. The 5-HT_1 receptor has been differentiated into 2 subtypes, known as 5-HT_{1a} and 5-HT_{1b}. They are distinguished by their preferential binding of indole derivatives and piperazine analogs, respectively. The two distinct types of responses to 5-HT application in the present study suggests heterogeneity within the cerebellar 5-HT receptor population. The ability of TFMPP to mimick the 5-HT mediated decrease in activity more specifically suggest the possible presence of 5-HT_{1b} receptors in the rat cerebellar granule cell layer.

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459.11 COMPLEXITIES OF SEROTONIN AND SEROTONIN ANTAGONIST INTERACTIONS MEASURED INTRACELLULARLY IN HIPPOCAMPAL CA_1 NEURONS. N. Gurevich, P.H. Wu* and P.L. Carlen. Playfair Neuroscience Unit, Toronto Western Hospital; Addiction Research Foundation; Depts. of Medicine (Neurology), Physiology and Pharmacology, Univ. of Toronto, Toronto, Ontario, Canada.

Serotonin (5-HT) when drop-applied to hippocampal CA_1 neurons in vitro produces an initial hyperpolarization and conductance increase with rapid onset and very slow (many minutes) recovery in cells not previously exposed to 5-HT. Subsequent 5-HT applications onto the same neuron cause a hyperpolarization of larger amplitude frequently followed within 10 sec to a few minutes by a depolarization which lasts up to 5 min and is associated with no conductance change or a slight decrease.

Several 5-HT antagonists were perfused or drop-applied onto the somatic region of the recorded neuron after control 5-HT responses were established. Methysergide (10^{-5}-10^{-3} M), a 5-HT_1 and 5-HT_2 receptor antagonist, Cyproheptadine (10^{-8}-10^{-6} M) and Mianserin (10^{-7}-10^{-4} M), both 5-HT_2 antagonists, all caused a moderate and long-lasting hyperpolarization by themselves. They increased the 5-HT depolarizing response and only methysergide decreased the 5-HT hyperpolarizing response in 50% of neurons examined. Drop-applied or perfused spiperone, a 5-HT_1 and 5-HT_2 antagonist, and ritancerin, a 5-HT_2 antagonist, caused a depolarization per se, blocked the 5-HT initial hyperpolarizing responses, and enhanced the later 5-HT depolarizing response. In summary, 5-HT receptor antagonists tested increased the later 5-HT depolarizing response and methysergide, spiperone, and ritancerin antagonized the early 5-HT hyperpolarizing response.

It is concluded that 5-HT produces complex electrophysiological responses on hippocampal CA_1 neurons. The long-lasting hyperpolarization induced by first 5-HT application appears to be desensitized and reveals a rapidly recovering hyperpolarizing response during the subsequent 5-HT applications. It is also concluded that there is some discrepancy between receptor binding studies and these 5-HT antagonist interactions with drop-applied serotonin measured electrophysiologically in hippocampal CA_1 neurons.

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459.12 INWARDLY RECTIFYING POTASSIUM CURRENTS INDUCED BY 5-HT IN DORSAL RAPHE J.T. Williams. Vollum Institute for Advanced Biomedical Research, Oregon Health Science University, Portland, OR 97201.

Intracellular recordings were made from neurons of the rat dorsal raphe in the slice preparation. Slices were maintained totally submerged in a flowing solution of physiological saline at 37°C. Drugs and solutions of differing ion content were applied by superfusion. Voltage clamp experiments were carried out using a single electrode voltage clamp amplifier. Neurons in the dorsal raphe were quiescent with a resting membrane potential near -60 mV. Neurons of the dorsal raphe used in the present study were characterized by (1) a hyperpolarization induced by 5-HT, 8 OH-DPAT or carboxyamido-5-HT that was antagonized by spiperone, (2) an inhibitory synaptic potential which reversed at the potassium equilibrium potential and was antagonized by spiperone and (3) a depolarization induced by noradrenaline which was blocked by prazosin. Between the membrane potentials of -60 and -100 mV the steady-state current/voltage plot was linear with a slope conductance of 4.9 ± 0.4 nS (17). The slope of the current/voltage plot increased in the presence of 5-HT agonists, and the 5-HT current reversed from outward to inward at the potassium equilibrium potential. The current induced by 5-HT agonists ($I_{5\text{-}HT}$) was determined at 10 mV intervals between -50 and -140 mV and these values were plotted as a function of membrane potential. The slope of the $I_{5\text{-}HT}$/voltage plot was not linear, increasing as the membrane potential was made more negative. The conductance increased caused by 5-HT ($G_{5\text{-}HT}$) at -60 mV was 2.0 ± 0.4 nS (5) and increased to 3.9 ± 1.0 nS (5) at -120 mV. In solutions of increased potassium ion content the reversal potential for the 5-HT current was shifted to less negative potentials and the 5-HT conductance increased at all membrane potentials. At -60 mV $G_{5\text{-}HT}$ increased from about 2 nS to 5.7 ± 1.7 nS (4) when the extracellular content of potassium was increased from 2.5 mM to 10.5 mM. The inward rectification of the 5-HT conductance is a mechanism by which the hyperpolarizing action of 5-HT is amplified. Such an inwardly rectifying potassium current has been described in other tissues following activation of several different receptor types and may represent a common ion channel which mediates inhibition.

459.13 THE EFFECT OF LYSERGIC ACID DIETHYLAMIDE ON SEROTONERGIC ELECTROPHYSIOLOGICAL RESPONSES IN RAT HIPPOCAMPAL SLICES. W.P. Clarke, S.G. Beck, and J. Goldfarb. Department of Pharmacology, Mount Sinai School of Medicine, CUNY, New York, NY 10029.

Superfusion of rat dorsal hippocampal slices with serotonin (5-HT) elicits at least two changes in the CA1 population spike evoked by stimulation of the stratum radiatum. The predominant effect, mediated by the 5-HT_{1A} receptor, is a rapid and reversible decrease in population spike amplitude (PSA). This decrease in the population spike is often preceeded by a transient increase in amplitude which becomes more apparent when the 5-HT_{1A} mediated decrease is blocked selectively e.g., with spiperone (Beck, S.G. et al., Eur. J. Pharmacol., 116: 195, 1985). The 5-HT induced increase is mediated by a receptor with pharmacological characteristics different from any of the known 5-HT receptor subtypes.

Superfusion of hippocampal slices with LSD (0.1-10uM) only decreased the CA1 PSA. The response was slow in onset, not rapidly reversible and was antagonized by preincubation with spiperone. LSD also reduced the decrease in PSA induced by 5-HT (3-10uM) when both drugs were administered concurrently. The apparently non-hallucinogenic analog, 2-bromo-LSD (BOL) (1-10uM) also decreased the PSA but was less potent than LSD.

In the presence of spiperone (10uM; 500 times it K_b at the 5-HT_{1A} site), 5-HT (1-10uM) produces only an increase in PSA. Even under these conditions, LSD (0.1-100uM) did not increase the PSA. Moreover, LSD did not appear to alter the increase produced by concurrent administration of 5-HT (3-10uM).

Based on these results we suggest that LSD is an agonist at the 5-HT_{1A} receptor that mediates the decrease in PSA in rat hippocampal slices, but LSD does not interact with the unclassified 5-HT receptor that mediates the increase in PSA. [Supported by grants DA-01875, DA-07315 and MH-41917].

459.14 SEROTONERGIC MODULATION OF FROG DORSAL ROOT GANGLION ACTION POTENTIALS: ANTAGONISM BY METHIOTHEPIN AND SPIPERONE. W. Marszalec and E.G. Anderson. Department of Pharmacology, University of Illinois at Chicago, Chicago, IL 60612

Serotonin (5-HT) reduces the width of tetraethylammonium-broadened (TEA) action potentials in frog dorsal root ganglion cells, presumably by reducing Ca^{++} influx (J. Neurosci. 6: 620, 1986). the putative 5-HT_{1A} agonist 8-OH dipropylaminotetralin (DPAT) widens these action potentials (Neurosci. Abstr., 337.5, 1986). To better understand the receptors involved, serotonin antagonists were tested for their effects on 5-HT and DPAT.

The superfusion of frog DRG cells with 5-HT in the presence of TEA reduced action potential duration in a dose-dependent manner. This effect was antagonized by 2 uM methiothepin (MTP):

5-HT Conc.	% Control (No MTP)	% Control (2 uM MTP)
0.1	84 ± 6 (n=9)	96 ± 2 (n=4)
1	51 ± 16 (n=19)	88 ± 12 (n=8)
10	49 ± 7 (n=6)	79 ± 14 (n=5)

This blocking effect persisted after one hour of washout and appeared to be non-competitive in that it could not be overcome with 100 uM 5-HT.

The putative 5-HT_{1A} antagonist spiperone (SPIP) at 10 uM also reduced the narrowing response of 5-HT:

5-HT Conc	% Control (No SPIP)	% Control (10 uM SPIP)
1	74 ± 3 (n=10)	96 ± 1 (n=7)
10	56 ± 6 (n=3)	84 ± 4 (n=3)

Unlike MTP, SPIP was reversed within 10 minutes of washout.

The widening effect of DPAT, was not antagonisted by either MTP (n=3) or SPIP (n=4). This failure should be tempered by the fact that 25 uM of DPAT was required whereas the concentrations of MTP and SPIP were limited to 4 and 10 uM respectively by their solubility.

Equimolar concentrations of the 5-HT_2 antagonist ketanserin failed to antagonize the response of either 5-HT or DPAT.

The efficacy of methiothepin in blocking the action of 5-HT is consistent with an action on 5-HT_1 receptors. The antagonism of 5-HT by spiperone suggests involvement of a 1A receptor subtype. However, this conflicts with the observation that DPAT, a high-affinity agonist for 1A sites, had the opposite effect of 5-HT and was not attenuated by SPIP nor MTP. This indicates that the receptor mediating the 5-HT response is different from that activated by DPAT. The high concentrations of DPAT required to obtain a response may indicate that DPAT binds to receptors with a lower affinity than the 1A site (5-HT_3?). This hypothesis is presently being tested. (Supported by USPHS Grant NS 17834).

459.15 AGE-DEPENDENT CHANGES IN SEROTONIN DORSAL RAPHE NEURONS: ELECTROPHYSIOLOGICAL ASSESSMENT IN FEMALE FISCHER 344 RATS. J.M. Lakoski and C.A. Richard*, Department of Pharmacology and Toxicology, Univ. of Texas Medical Branch, Galveston, TX 77550.

Alterations in both hormone and neurotransmitter systems have been identified to underlie a variety of age-related changes in neuroendocrine function, including the decline of female reproductive function. Specific age-related changes in central serotonin (5HT)-containing neuronal systems in the aging female rat have been identified from neurochemical and behavioral studies. However, studies of cellular physiological events which may mediate age-related changes in reproductive function, particularly with respect to 5HT neurons, are lacking. Therefore, as a prelude to studies evaluating age-related changes in sensitivity to microiontophoretically applied 5HT agonists in the dorsal raphe nucleus (DRN), we have evaluated whether spontaneous 5HT DRN cell firing is altered in the aging female rat.

Intact 18 mo and 28 mo old female Fischer 344 rats were anesthetized with chloral hydrate and utilized for extracellular recording studies of 5HT neurons in the DRN. Cycling young (3-4 mo), middle-aged (11-12 mo) and acyclic (18 mo) females ovariectomized at least one week prior to recording were also utilized to provide a constant level of circulating plasma estradiol comparable to that of the intact aged rats. Population sampling of spontaneously active 5HT neurons in the DRN revealed no significant differences in the number of cells encountered (3 passes/animal) with respect to age. However, a significant age-related decline in the average firing rates of 5HT cells sampled was apparent: 3 mo (10.36 ± 0.82 sp/10 sec, n=61), 11 mo (8.76 ± 0.64 sp/10 sec, n=64), 18 mo (7.29 ± 0.57 sp/10 sec, n=75) and 28 mo (4.90 ± 0.84 sp/10 sec, n=21) groups. Significant declines in the range of firing rates observed with respect to aging were identified by statistical comparison (Mann-Whitney U) of rankings between age groups.

These data have identified specific age-related changes in 5HT cell function in the DRN that may underlie neuroendocrine events associated with female reproductive aging. Studies are presently underway to determine whether the pharmacologic profile of autoregulatory mechanisms in the DRN change with respect to age. Supported by NIH AG-06017.

459.16 ENHANCED INHIBITORY RESPONSES OF SEROTONIN NEURONS IN THE DORSAL RAPHE NUCLEUS (DRN) AFTER REPEATED COCAINE EXPOSURE. K.A. Cunningham, E.K. Asprodini, N.A. Bernau, C.A. Richard*, and J. M. Lakoski. Dept. Pharmacology/Toxicology, Univ. Texas Medical Branch, Galveston, TX 77550.

A potent stereoselective inhibitory effect of cocaine on serotonin (5-HT) cell firing in the DRN has been described after both systemic and microiontophoretic application. We hypothesized that alterations in the responsivity of these neurons may also develop upon long-term cocaine administration; such effects may be relevant to the gradual development of behavioral sensitization in animals and psychosis in humans. We have investigated the pharmacological profile of 5-HT neurons recorded in the rat DRN after two regimens of chronic cocaine administration (i.p.): (1) 10 mg/kg once daily for 10 days; (2) 15 mg/kg/injection twice daily for 7 days. After either regimen of repeated cocaine or saline injections, we assessed the ability of cocaine (0.125-8.0 mg/kg, i.v.) or the 5-HT autoreceptor agonist 8-hydroxy-2-(di-n-propyl-amino)-tetralin (8-OHDPAT; 0.31-20 µg/kg, i.v.) to depress the firing rate of 5-HT neurons recorded extracellularly in the DRN; recordings were conducted within 24-26 hours after the final treatment injection. The daily (10 mg/kg) schedule did not significantly alter the ability of cocaine to inhibit the spontaneous activity of 5-HT neurons. In contrast, following the twice daily cocaine (15 mg/kg) treatment, the dose-response curves for both cocaine and 8-OHDPAT inhibitory responses were shifted 2-fold to the left of control. The dose of cocaine required to inhibit cell firing by 50% in cocaine-treated rats (ID_{50}=0.78 ± 0.18 mg/kg, N=12) was reduced as compared to rats treated with saline (ID_{50}=1.39 ± 0.26 mg/kg, N=13); this trend approached significance (p=0.07). The ID_{50} of 8-OHDPAT, however, was significantly reduced after repeated cocaine exposure (ID_{50}=1.33 ± 0.28 µg/kg, N=8) when compared to controls (ID_{50}=4.40 ± 1.31 µg/kg, N=8; p<0.05). The mean dose of 8-OHDPAT required to completely depress cell firing was also significantly reduced after cocaine (5.0 ± 1.2 µg/kg, N=8) vs. saline treatment (11.4 ± 2.7 µg/kg, N=8; p<0.05). In summary, chronic cocaine treatment appears to alter the pharmacological profile of DRN 5-HT neurons. Although further research is required to identify the mechanisms underlying the observed effects, the enhanced inhibitory response to acute (i.v.) injections of cocaine and 8-OHDPAT suggests that modifications of DRN 5-HT autoregulatory mechanisms have developed as a result of chronic cocaine exposure.

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459.17 THE NORADRENALINE-β-PHENYLETHYLAMINE INTERACTION IN THE RAT CEREBRAL CORTEX: A POST-SYNAPTIC EFFECT? I.A. Paterson* (SPON: P.V. Sulakhe). Psychiatric Research Div., Saskatchewan Health, CMR Bldg., Univ. of Saskatchewan, Saskatoon, Sask., S7N OWO, Canada.

Recent work has suggested that noradrenaline (NA) may, in part, mediate the central effects of the endogenous trace amine, β-phenylethylamine (PE), and it has been demonstrated that PE causes an increase in responses to iontophoretically applied NA. The present experiments were carried out to determine the probable mechanism of this interaction.

Male Wistar rats (220-250 g) were subject to one of the following pretreatments; none (control animals), reserpine (10 mg/kg, i.p., 24 hours) or vehicle (20% ascorbic acid, 2 ml/kg), α-methyl-p-tyrosine (AMPT, 250 mg/kg, 30 minutes before anaesthesia) or vehicle (saline, 1 ml/kg), or unilateral electrolytic lesion of the locus coereleus (LC) 14-21 days prior to recording. The rats were anaesthetised with urethane (1.2 g/kg, i.p.) and six-barrelled glass microelectrodes were introduced into the cerebral cortex through a burr hole in the skull. Standard extracellular recording and iontophoresis circuits were used. Spontaneously active units were recorded 400-1200 μm below the pial surface.

In control animals 81% of the neurones responded to NA, and 66% to PE. Small currents of PE (0-6 nA) increased responses to NA to between 155% and 360% of control response size, without affecting the baseline firing rate, or responses to GABA or 5-hydroxytryptamine. Pretreatment with AMPT, saline or 20% ascorbic acid had no effect on the responsiveness of neurones to PE or NA, nor did it affect the enhancement of NA responses by PE. Cortical neurones contralateral to LC lesions responded in the same way as neurones in control conditions. In animals pretreated with reserpine, and in the cerebral cortex ipsilateral to LC lesions, no responses to PE were seen, though neurones responded in the normal manner to NA. Even though neurones would not respond to PE alone, PE still induced increases in responses to NA.

Treatments which deplete the granular stores of NA and damage noradrenergic presynaptic terminals (reserpine and LC lesions) blocked responses to PE. This provides further evidence that central effects of PE are mediated by NA. Inhibition of NA synthesis by AMPT had no effect in the short term, indicating that the responses to PE are dependant on the granular stores of NA. The fact that none of the treatments altered the PE-induced enhancement of NA responses demonstrates that this interaction is not dependant on intact, functioning presynaptic noradrenergic terminals. This leaves a post-synaptic effect of PE as the most likely mechanism of this interaction. This supports the hypothesis that certain trace amines may modulate catecholamine neurotransmission.
(Supported by Saskatchewan Health and the Saskatchewan Health Research Board).

459.18 PROPRANOLOL BLOCKS THE INHIBITION OF CEREBELLAR PURKINJE CELLS BY SEROTONIN AND PUTATIVE 5-HT_{1A} AGONISTS. H.K. Strahlendorf, J.C. Strahlendorf, E.J. Darrow and M. Lee. Depts. of Neurology and Physiology, Sch. of Med., Texas Tech University Health Sci. Ctr., Lubbock, TX 79430.

Previous attempts to antagonize serotonin (5-HT) mediated inhibition of cerebellar Purkinje cells (PCs) have been relatively unsuccessful (Exp. Br. Res. 56:50, 1984) with the exception that the chloride ionophore blocker picrotoxin and the mixed 5-HT_{1A}/5-HT_2 antagonist spiperone are somewhat effective (Exp. Neurol. 1987 In Press, and Soc. Neurosci. Abst. 12:1238, 1986). Recently several lines of evidence suggest that the beta adrenergic antagonist propranolol (PRO) stereoselectively binds to 5-HT_{1A} receptors and blocks neuronal inhibition elicited by compounds which act on 5-HT_{1A} receptors (Eur. J. Pharm. 101:289, 1984 and Eur. J. Pharm. 128:295, 1986). We have assessed the ability of (+) and (-) isomers of PRO to block inhibition of PCs by 5-HT and putative 5-HT_{1A} receptor agonists in urethane anesthetized rats. Extracellular single cell recordings of PCs were made using 5-barrel iontophoretic pipettes filled with 5-HT creatinine sulfate, 8-hydroxy-2-(di-n-propylamino) tetralin HBR (8-OH-DPAT), (-) PRO HCl and (+) PRO HCl. PCs were identified by standard electrophysiologic criteria including the occurrence of complex spikes.

5-HT and 8-OH-DPAT elicited inhibition of PC firing; at equivalent ejection currents 8-OH-DPAT was frequently more effective than 5-HT. Relatively high currents of 8-OH-DPAT (>40nA) often produced apparent non-specific membrane effects that summed with repeated applications. Both isomers of PRO elicited profound membrane effects when applied continuously for periods in excess of 1 minute even at low (<10nA) ejection currents. Often apparent membrane actions of 8-OH-DPAT and isomers of PRO were additive to the extent of completely suppressing cell firing. (-) PRO effectively antagonized 5-HT mediated inhibition in 6 out of 8 neurons (75%); however, only 2 cells (25%) demonstrated reasonable recovery of 5-HT effects. Average reduction of 5-HT inhibition for these 2 cells was 51%. (-) PRO also antagonized inhibition elicited by 8-OH-DPAT in 7 out of 9 cells (78%); 5 cells demonstrated recovery after PRO. Average antagonism of 8-OH-DPAT mediated inhibition was 53%. (+) PRO reduced inhibition to 5-HT in 2 out of 8 cells (25%) and to 8-OH-DPAT in 1 out of 6 cells (17%). (+) PRO antagonized 5-HT effects by an average of 49%; whereas 8-OH-DPAT actions were reduced by only 12%. These data indicate that (-) PRO is more effective in antagonizing 5-HT and 8-OH-DPAT suppression of PC firing. Qualitatively, (-) PRO may prove to be more effective against 8-OH-DPAT effects than against inhibition by 5-HT (8-OH-DPAT 5/9, 56%; 5-HT 2/8, 25%). Moreover, inhibition of PCs by 5-HT may involve mechanisms in addition to 5-HT_{1A} receptors. (Supported by NIH Grant ROI NS 19296)

459.19 CHARACTERIZATION OF RECEPTOR SUBTYPES MEDIATING INHIBITION OF PURKINJE CELL SPONTANEOUS FIRING BY SEROTONIN IN THE IN VITRO CEREBELLAR SLICE. E. J. Darrow, H. K. Strahlendorf, and J. C. Strahlendorf. Depts. of Physiology and Neurology, Texas Tech Univ. Health Sci. Center, Sch. of Med., Lubbock, TX 79430.

In previous reports (Darrow, et al., Soc. Neurosci. Abst. 16: 1238, 1986) we have shown that cerebellar Purkinje cells (PC) simple spike (SS) activity, in the in vitro slice preparation, responds to microiontophoretic application of serotonin (5-HT) in a manner qualitatively and quantitatively similar to results obtained in situ. The observed responses were of three types: pure inhibition, pure excitation, and a biphasic response of inhibition followed by excitation. Furthermore, using putative selective 5-HT receptor subtype agonists and antagonists in the in vitro slice preparation we have obtained data consistent with the hypothesis that cerebellar PCs have multiple 5-HT receptor subtypes.

In our current studies we are examing the effects of the 5-HT_{1B}/5-HT_2 specific agonist 1-(3-trifluoromethyl-phenyl)-piperazine (TFMPP) and the 5-HT_2 specific agonist 1-(2,5-dimethoxy-4-iodo-phenyl)-2-aminopropane (DOI) on PC SS activity. These compounds were iontophoretically applied to PCs superfused with control media and media containing 1-5 μm concentrations of ketanserin. Our preliminary results show that in vitro PC responses to iontophoresis of TFMPP are qualitatively similar to 5-HT, with inhibitory, excitatory and biphasic responses. The responses to TFMPP, however, appear to be considerably smaller than the responses elicited by 5-HT. The inhibitory response produced by TFMPP appears to be dose-dependent over ejection currents of 5-80 nA, and is not blocked by ketanserin superfusion. Iontophoresis of DOI at currents from 5-40 nA on the other hand, does not appear to produce any consistent effect on SS activity. While there appears to be a tendency toward inhibition of SS activity, there is no significant dose-response relationship. Superfusion of the slices with ketanserin was ineffective in blocking responses elicited by DOI. Thus, it appears that the inhibitory response elicited by DOI is non-specific in nature. Results of this and previous studies suggest that both 5-HT_{1A} and 5-HT_{1B} receptor subtypes may partly mediate an inhibitory effect on PC SS activity. The 5-HT_{1B} subtype may also be involved in excitatory responses. The inconsistent effects elicited by the DOI and ketanserin suggests that PCs lack the ability to respond to these 5-HT_2 specific compounds in a manner demonstrable by extracellular recordings. (Research funded in part by the Tarbox Parkinson's Disease Institute, TTUHSC).

459.20 SEROTONIN MODULATION OF GABA-MEDIATED INHIBITION ON CEREBELLAR PURKINJE CELLS INVOLVES $GABA_A$ AND $GABA_B$ RECEPTOR ACTIVATION. J. C. Strahlendorf, H. K. Strahlendorf, and M. Lee. Depts. of Physiology and Neurology. Texas Tech Univ. Hlth. Sci. Ctr., Sch. of Med., Lubbock, Texas 79430.

In previous studies, we have found that inhibitory effects of iontophoretic application of serotonin (5-hydroxytryptamine, 5-HT) on cerebellar Purkinje cells (PCs) were antagonized significantly by continuous application of the chloride ionophore antagonist picrotoxin (Lee, M. et al., Exp. Neurol. in press). We also have shown that iontophoretic applications of pentobarbital and phenobarbital potentiated the magnitude and prolonged the duration of 5-HT-mediated inhibitions, and converted 5-HT mediated excitations primarily to inhibitions (Strahlendorf,J. et al., submitted). Since our previous studies have suggested a potential interplay between the cerebellar GABAergic and serotonergic systems, we have assessed the effects of 5-HT on inhibitions elicited by GABA and GABA agonists on PCs in urethane anesthetized rats.

Iontophoretically applied 5-HT modulated GABA-mediated inhibition in a biphasic manner which consisted of an initial decrease followed by an augmentation of GABA-mediated inhibition. Specifically, 81% (72/89 cells) of PCs initially showed an average 48% decrease in their inhibitory response to pulsatile GABA in the presence of 5-HT. During the secondary phase of the biphasic response, 61% of PCs responded to continuous 5-HT with an average 173% increase in their inhibitory response to GABA (onset time averaging 8 minutes). Whereas serotonin augmented GABA inhibition in 61% of PCs during the secondary phase, 32% of the cells tested showed an average 58% attenuation of GABA mediated inhibition in the presence of 5-HT. The differences in the response to 5-HT appears to be related to the initial firing rates of the PC. To discern which GABA receptor 5-HT may be linked to, studies identical to those involving pulsatile applications of GABA were initiated using muscimol, a $GABA_A$ agonist, and baclofen, a GABAB agonist. During the initial phase, inhibitory responses of baclofen were inhibited in 63% of the cells by an average of 48%, whereas 5-HT augmented baclofen responses in 56% of PCs by an average 232% during the secondary phase (n=58). On the other hand, inhibitory responses of muscimol were initially augmented by an average of 74% in 58% of the cells tested, and in 77% of PCs muscimol actions were augmented during the secondary phase by an average of 191% (n=43). These findings suggest that serotonin directly modulates GABA mediated inhibition of PCs via an action on both $GABA_A$ and $GABA_B$ receptor subtypes (Supported by NIH Grant ROI NS 19296).

460.1 SOMATOSTATIN'S EFFECT ON MUSCARINIC RECEPTOR-LINKED PHOSPHOINOSITIDE TURNOVER IN THE BRAIN. S. Kito, R. Miyoshi and M. Shimizu*. Third Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan.

In our previous studies, it was noticed that somatostatin affected oxotremorine binding in the rat hippocampus whose muscarinic acetylcholine receptors were dominantly of the M1 type. It has been proposed that central M1 receptors are strongly linked to phosphatidylinositol (PI) turnover. Basing on this fact, the authors investigated the effect of somatostatin on carbachol-stimulated PI turnover using rat hippocampal slices. Phosphoinositide hydrolysis was measured according to the method of Berridge et al. As the results, somatostatin caused a further increase of carbachol-induced ^{3}H-inositol-1-phosphate (IP_1) accumulation in dose-dependent manner. Such effect of somatostatin was significantly elevated when we used somatostatin-28 instead of [D-trp^{8}] somatostatin. Furthermore, the investigations were done on how either protein kinase C activator or inhibitor modified this somatostatin's effect. H-7, a protein kinase C inhibitor, not only increased carbachol-induced accumulation of ^{3}H-IP_1 by itself, but also exaggerated somatostatin's augmented effect to the extent more than the additive effect. On the other hand, 1-oleoyl-2-acetyl-glycerol inhibited carbachol-induced ^{3}H-IP_1 accumulation with the preservation of the somatostatin's enhancing effect. This means the existence of a feedback mechanism between protein kinase C and PI turnover. There have been evidences to support the fact that PI turnover is also linked with GTP binding protein. In our experimental system, islet-activating protein (IAP) was intraventricularly injected 4 days prior to prepare hippocampal slices. It was noticed that somatostatin's effect on carbachol-induced ^{3}H-IP_1 accumulation had gone. To analyse these results more precisely, the time course of carbachol-induced accumulation of ^{3}H-inositol phosphates was studied and we observed that both IP_2 and IP_3 reached peak values much slower (10 minutes) in the cerebral cortex than in other non-neuronal tissues. Somatostatin's modulating effects on M1 receptors were further investigated taking this kinetic characteristics into considerations.

460.2 ADENYLATE CYCLASE ACTIVATION IS NOT SUFFICIENT TO STIMULATE SOMATOSTATIN RELEASE FROM DISPERSED CEREBRAL CORTICAL AND DIENCEPHALIC CELLS IN GLIA-FREE CULTURES. L. TAPIA-ARANCIBIA, N. PARES-HERBUTE*, H. ASTIER*, S. REICHLIN[1] and J. NATHANSON[2]. Dept. of Neuroendocrinology, University of Montpellier II, F-34060 Montpellier. [1]Endocrine Division, Tufts New England Medical Center Boston 02111 and [2]Dept. of Neurology, Mass Gen. Hosp., Boston 02114.

We have previously shown that VIP stimulates somatostatin release from rat cerebral cortical and diencephalic whole cell cultures (Brain Res.,336:67,1985). In this study we have investigated the mechanisms involved in the somatostatin release using dispersed rat cerebral cortical and diencephalic cells in glia-free cultures.

An early (after 3 days in culture) exposure of cells to cytosine arabinoside (10 µM for 48 h)allows us to obtain on the 10th day of culture, a highly purified preparation of neurons as revealed by a weak (5 %) immunohistochemical staining for glial fibrillary acid protein (GFAP) and vimentin, two markers of astrocytes.

Under these conditions when VIP induces somatostatin release from cortical and diencephalic neuronal cultures,the peptide also causes large increases in intracellular cyclic AMP.Both the release of somatostatin and the increase in cyclic AMP elicited by VIP are time and dose-correlated,require exogenous calcium,can be blocked by cobalt ion and can be qualitatively mimicked by depolarizing concentrations of potassium ion (30 µM).In contrast,a direct activation of adenylate cyclase by forskolin (from 10^{-8}M to 10^{-4}M) causes a large and dose-dependent increase in cyclic AMP content but does not induce somatostatin release. When VIP (1 µM) and forskolin (100 µM) were added simultaneously to the incubation media,the intracellular cyclic AMP accumulation was additive but somatostatin release was not significantly different from that induced by VIP alone.These results suggest that cyclic AMP might not be the second messenger of VIP effect on the somatostatin secretion process and indicate that VIP-stimulation of cyclic AMP formation and VIP-stimulation of somatostatin release are two phenomena which could be dissociated. The calcium ionophores ionomycin and A23187 also stimulate somatostatin release and increase intracellular cyclic AMP in a dose-dependent manner.We then evaluated the effects of a phorbol ester (Phorbol 12-myristate 13 acetate, PMA) which act through the phosphoinositide pathway.In the absence of VIP,PMA alone elicited a dose-dependent (from 10^{-9}M to 10^{-5}M) increase in somatostatin release.These data taken together indicate that 1/cyclic AMP formation is not a prerequisite condition for somatostatin release 2/Ca^{2} entry into the cells is able to provoke both somatostatin release and cyclic AMP accumulation in a dose-dependent manner 3/PMA also stimulates somatostatin release and this effect is not additive with that obtained at saturating concentrations of VIP.

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460.3 EFFECTS OF GUANINE NUCLEOTIDES ON THE INCREASE IN BETA-ADRENERGIC RECEPTOR BINDING CAUSED BY LESIONS OF CENTRAL SEROTONIN (5-HT) NEURONS. C.A. Stockmeier, D. Fox* and K.J. Kellar. Dept. of Psychiatry, Case Western Reserve Univ. Sch. of Medicine, Cleveland, OH 44106; Dept. of Pharmacology, Georgetown Univ. Sch. of Medicine, Washington, DC 20007.

Selective lesions of central 5-HT neurons increase the number of beta-adrenergic receptors in rat frontal cortex and hippocampus (Science 230:323, 1985). We have measured the effects of 5'-guanylylimidodiphosphate [Gpp(NH)p] on beta-receptor binding following 5,7-dihydroxytryptamine (5,7-DHT)-induced lesions of 5-HT neurons. In addition, the in vitro affinity of L-isoproterenol (L-Iso) for the beta-receptor in cortex was determined in control and lesioned rats. Anesthetized rats were pretreated with desipramine and 5,7-DHT or saline was injected into both the dorsal and median raphe nuclei (7ug free base), or bilaterally into the lateral ventricles (125 ug free base). Two weeks after the ventricular lesion and 7 weeks after the raphe lesion the content of 5-HT was reduced by 95% in the frontal cortex while the content of norepinephrine was not significantly affected.

The ventricular lesion resulted in a doubling of [^{3}H]dihydroalprenolol ([^{3}H]DHA) binding to beta-receptors in the hippocampus. The lesion-induced increase in binding was completely prevented by 100 uM Gpp(NH)p in vitro, while binding in control tissues was unaffected by Gpp(NH)p. Specific binding was defined with either 200 uM L-Iso or 10 uM dl-propranolol. The raphe lesion produced a 77% increase in [^{3}H]DHA binding in the hippocampus and no change in the cerebellum. [^{3}H]DHA binding in the thalamus was enhanced 48% by the raphe lesion, and again the increase in binding was prevented by Gpp(NH)p in vitro. In some way the 5-HT lesion appears to change the sensitivity of antagonist-labeled beta-adrenergic receptor binding to guanyl nucleotides.

The in vitro affinity of L-Iso for the beta-receptor in cortex was decreased 3 fold by the raphe lesion. The Hill slope of the competition curves was also reduced by the raphe lesion from 0.68 to 0.38, suggesting an increase in a binding site with low affinity for L-Iso. Non-linear regression analysis of the competition curves in control cortex revealed a high and low affinity binding site (K_I= 34 nM and 13 uM, respectively) with the number of high affinity sites predominating by about 8:1. In cortex from rats with the raphe lesion the high-affinity sites outnumbered the low affinity sites by only about 2:1. These data suggest that the lesion of central 5-HT neurons induces an increase predominantly in the number of beta-adrenergic receptors with low affinity for L-Iso.

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460.4 COMPARISON OF BACLOFEN AND PHORBOL ESTERS AS AUGMENTORS OF ISOPROTERENOL-STIMULATED CAMP PRODUCTION IN RAT BRAIN SLICES. R.W. Scherer*, E.W. Karbon*, J.W. Ferkany and S.J. Enna. Nova Pharmaceutical Corporation, Baltimore, Maryland 21224-2788.

Stimulation of $GABA_B$ receptors by baclofen (BAC) or GABA augments cAMP accumulation in cerebral cortical slices in the presence of isoproterenol (ISO). Likewise, tumor-promoting phorbol esters (PE) augment cAMP accumulation in cortical slices, suggesting that $GABA_B$ agonists may act through activation of protein kinase C. However, the actions of PE and BAC in augmenting the cAMP response to ISO differ in some ways. For example, the action of BAC, but not PE, is reduced by EGTA. Furthermore, PE potentiates forskolin-stimulated cAMP production in brain slices, whereas BAC inhibits it. The present study further compares the actions of BAC and PE in an attempt to establish whether or not these substances share common mechanisms with regard to their effect on cAMP accumulation.

For this study, rat cerebral cortical slices (260 µm x 260 µm) were preincubated (60 min) with [^{3}H]-adenine, after which BAC (100 µM) or phorbol 12-myristate, 13-acetate (PMA; 10 µM) was added and the incubation continued for an additional 10 - 15 min. The slices were then exposed to ISO for 10 min, the reaction terminated, and the percent conversion (%) of [^{3}H]-adenine to [^{3}H]-cAMP determined.

Co-exposure of slices to saturating concentrations of PMA and BAC resulted in a greater augmentation of the ISO response than was obtained with either agent alone (ISO = 0.35%; ISO/BAC = 1.76%; ISO/PMA = 2.21%; ISO/PMA/BAC = 3.42%). Although the protein kinase C inhibitor H7 (100 µM) did not alter basal or ISO-stimulated cAMP accumulation, pretreatment of slices with H7 reduced PMA- but not BAC-induced augmentation of second messenger accumulation (ISO = 0.66 ± 0.14%; ISO/PMA = 1.33 ± 0.24%; ISO/PMA/H7 = 0.73 ± 0.15%; ISO/BAC = 1.98 ± 0.26%; ISO/BAC/H7 = 2.10 ± 0.21%). When protein kinase C was desensitized by a 3 hour exposure to PMA, the cAMP augmenting response to PE was completely abolished, whereas that to BAC was unchanged.

Previous reports[(1)] have suggested that BAC and PMA augment ISO-induced cAMP conversion by means of different mechanisms. The results of the present study showing that (1) H7, a protein kinase C inhibitor, can inhibit PMA-, but not BAC-induced augmentation; (2) the response to PMA but not BAC, is reduced following desensitization of protein kinase C; and (3) the augmenting responses to PMA and BAC are additive, support this proposal. Thus, it would appear that PMA, but not BAC, augments ISO-stimulated cAMP accumulation in rat brain slices by activating protein kinase C.

1. Karbon, E.W. et al. J. Neurochem. 47: 1566-1575, 1986.

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460.5 CONVERGENCE OF NEUROMODULATOR ACTIONS THROUGH DIFFERENT SECOND MESSENGER SYSTEMS IN CORTICAL NEURONS. R.C. Foehring, P.C. Schwindt, and W.E. Crill. Dept. of Physiology and Biophysics, Univ. of Washington Sch. of Med., Seattle, WA 98195.

Layer V neurons from cat sensorimotor cortex were studied in an *in vitro* slice preparation using intracellular recording and single electrode voltage clamp (SEVC). We observed the modification of repetitive firing behavior and subsequent afterhyperpolarizations (AHPs) by neuromodulators. Following adequate stimulation (sustained repetitive firing or depolarizing SEVC step) these neurons exhibited a long-lasting AHP (sAHP) lasting 3-30s. Both norepinephrine (NE) and muscarine selectively reduced this sAHP and the underlying ionic currents in a dose-dependent manner. The NE effect was mediated via β-1 adrenoreceptors. The effects of NE and muscarine added at low doses but occluded at higher doses, suggesting convergence of action at some point after binding to their receptors. Consistent with activation of the adenylate cyclase system, the NE effect was mimicked by extracellular application of dibutyryl-cAMP, 8-bromo-cAMP, or forskolin. In platelets, SQ22,536 (Squibb) inhibits adenylate cyclase activity (Harris et al. J. Cycl. Nucl. Res. 5:125). Preliminary data suggests that SQ22,536 can prevent NE from decreasing the sAHP. SQ22,536 does not prevent muscarine from reducing the sAHP, suggesting that muscarine does not act via the adenylate cyclase system, but rather through another pathway. We hypothesize that muscarine acts via the phosphatidyl inositol cycle, with activation of protein kinase C mediating the reduction of the sAHP. Further, we predict that the convergence of the effects of NE and muscarine occurs at, or after the phosphorylation step. We are currently testing these hypotheses.

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460.6 RECEPTOR AND IONOPHORE REGULATION OF CA^{++}-DEPENDENT PHOSPHORYLASE ACTIVITY AND MYOSIN LIGHT CHAIN PHOSPHORYLATION IN BC3H-1 MUSCLE CELLS. R.D. Brown*, D.A. Taylor*, and S. Toranji*. (SPON: J. Herz) Dept. of Pharmacology, UC San Diego, La Jolla, CA 92093 and Dept. of Pharmacology, UT Health Sci. Ctr. at Dallas, Dallas, TX 75235.

We have examined Ca^{++}-dependent intracellular responses upon stimulation of BC3H-1 muscle cells by norepinephrine (NE), histamine (HIS), or the Ca^{++} ionophore A23187. These cells possess $_1$-adrenergic and H1-histaminergic receptors which are functionally coupled to intracellular Ca^{++} mobility. (R.D. Brown, et al., Mol. Pharmacol. 29:531, 1986) Activation of glycogen phosphorylase to the active a form was monitored as a Ca^{++}-dependent metabolic response. Phosphorylation of myosin 20 kD light chain (P-20) was measured by Western blot techniques as an indicator of Ca^{++}-dependent activation of contractile proteins. The phosphorylase activity ratio, a/(a+b), was determined as the fraction of the active a form relative to the total enzyme activity (a+b forms) measured with the activator adenosine 5'-monophosphate. This ratio in resting BC3H-1 cells is 0.39±.03. Maximally effective concentrations of NE (3uM), HIS (100 uM), or A23187 (10 uM) activate phosphorylase a activity to ratios of 0.8-0.9 within 5 sec. The enzyme remains fully activated during 20 min exposure to either NE or A23187, whereas enzyme activity returns to control values within 5 min during exposure to HIS. The sustained activation by NE is reversed upon addition of the antagonist phentolamine. Stimulation by either agonist or ionophore also results in rapid myosin P-20 phosphorylation. Resting values of 0.12±.05 mole P/mole light chain increase to maximum values of 0.3-0.5. Although some differences in kinetics are observed, all three agents elicit peak phosphorylation within 30 sec; phosphorylation returns to basal values within 2-5 min. Thus, activation of Ca^{++}-dependent responses in BC3H-1 cells is rapid in onset. During prolonged agonist exposure, $_1$-adrenergic receptors elicit sustained phosphorylase activation whereas H1-histamine receptors desensitize. Moreover, myosin phosphorylation returns to basal values during persistent phosphorylase activation, indicating that these two processes couple inequivalently to prolonged alteration in intracellular Ca^{++}. Supported by American Heart Assoc., California Affiliate (84-S105; RDB, ST) and by NIH (HL 26930, DAT).

460.7 EFFECTS OF NORADRENERGIC DEAFFERENTIATION ON NEURONAL RESPONSIVENESS TO BIOGENIC AMINES IN CEREBRAL CORTEX: A MICROIONTOPHORETIC STUDY IN THE RAT. Jean Goyer* and André Ferron. (SPON: H.H. Jasper) Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, Montréal, Canada.

Recent research has provided a wealth of information pointing to the existence of interactions between the three biogenic amines, noradrenaline (NA), dopamine (DA) and serotinin (5-HT) in the cerebral cortex. To further investigate these interactions, the responsiveness of unitary fronto-parietal cortical neurons to microiontophoretic applications of NA, DA and 5-HT was assessed in urethane-anesthetized adult male Sprague-Dawley rats, 2 to 4 weeks after bilateral destruction of NA afferents with 6-hydroxydopamine (6-OHDA, 8 µg in the dorsal NA bundle). The endogenous NA cortical contents, assayed by HPLC, showed an 80% reduction (Reader and Brière, Br Res Bull. 11: 687, 1983), while endogenous DA and 5-HT were not significantly changed, suggesting a sparing of those fibers. The effects of biogenic amines on spontaneously active cortical neurons was assessed with the $I.T_{50}$ method, i.e. by measurement of the current x time (nA x sec) required to obtain a 50% depression of firing rate. Two weeks after 6-OHDA treatment, the responsiveness to all three amines was increased, as indicated by a corresponding lowering of $I.T_{50}$ values (see table). Four weeks after treatment, DA sensitivity was back to normal, while both NA and 5-HT sensitivities remained above control values. These results indicate that, in rat cerebral cortex, NA deafferentation initially induces not only a NA hypersensitivity, but also an increased sensitivity to DA and 5-HT. The recuperation of a normal responsiveness of cortical neurons to DA suggests that this system possesses an adaptive capability. Several hypotheses could account for 5-HT hypersensitivity, occuring either presynaptically in the raphe (diminution of 5-HT synthesis rate due to NA depletion) or cortical terminals (increase in 5-HT autoreceptor sensitivity) or postsynaptically in cortical cells (coupling of NA and 5-HT receptor synthesis rates). The data is in keeping with the current hypothesis of a functional interregulation between cortical NA, DA and 5-HT systems.

I.T50 (in nC)

	CONTROL	2 WEEKS	3 WEEKS	4 WEEKS
NA	1502	401	578	556
DA	1265	601	974	1221
5-HT	970	86	87	214

(Supported by Medical Research Council of Canada).

460.8 NEURONAL RESPONSIVENESS TO BIOGENIC AMINES IN ADULT RAT CEREBRAL CORTEX AFTER NA DEAFFERENTIATION AT BIRTH. Pascale Ouellet* and André Ferron, Centre de recherche en sciences neurologiques (Dép. de physiologie), Université de Montréal, Montréal, Canada.

Recent studies have shown that the administration of 6-hydroxydopamine (6-OHDA) in neonate rat induces a selective denervation of the cortical noradrenergic innervation, sparing the dopaminergic and serotonergic nerve terminals. The present microiontophoretic studies were carried out to assess the responsiveness of cortical neurons to noradrenaline (NA), dopamine (DA) and serotonin (5-HT), after selective lesioning of NA system. NA deafferentation was produced by 3 subcutaneous injections of 6-OHDA (100 µg/kg) at day 0 (day of birth), day 1 and day 2. The responsiveness to biogenic amines was evaluated by the IT_{50} method i.e. measurement of the current (in nA) X time (sec) required to obtain a 50% depression of firing rate. The effect of these transmitters on the spontaneously active neurons was examined in the sensorimotor cortex of urethane-anesthetized rats. A vast majority of spontaneously active neurons in the sensorimotor cortex responded by a depression of their firing rate to all or either one of the biogenic amines. Two to 3 months after 6-OHDA denervation, responsiveness to NA was enhanced (460 vs 1397 nC). In contrast, responsiveness to 5-HT was markedly reduced (1499 vs 680 nC). There were no significant differences in IT_{50} values for DA (2141 vs 1784 nC). In a second series of experiments, selectives NA agonists, phenylephrine (PHE, alpah-1), clonidine (CLO, alpha-2) and isoproterenol (ISO, beta), were administered by microiontophoresis in control and 6-OHDA-treated rats to determine which adrenoceptors were involved in the denervation supersensitivity. In 6-OHDA-deafferented cortex, the responsiveness to PHE was increased (1358 vs 2898 nC) whereas the responsiveness to CLO was decreased (2048 vs 1261 nC). The sensitivity of beta adrenoceptors was unchanged (2280 vs 2652 nC). These data indicate that, in the cerebral cortex of adult rats treated with 6-OHDA at birth, the increased responsiveness to NA was mainly imputable to the development of an alpha-1 receptor supersensitivity. The decreased sensitivity to 5-HT following NA deafferentation suggests a functional interaction between the NA and 5-HT cortical systems.

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460.9 NOREPINEPHRINE REGULATION OF SEROTONIN RELEASE IN SPINAL CORD. D.J. Jones (SPON: J.W. Maas). Depts. of Anesthesiology and Pharmacology, Univ. Texas Hlth. Sci. Ctr., San Antonio, TX 78284-7838

Previous studies have established that descending spinal cord serotonin (5-HT) and norepinephrine (NE) pathways influence cardiovascular, motor and pain reflexes at segmental levels. These pathways terminate at all spinal levels and anatomical studies have demonstrated common localization of terminals in the spinal grey. Starke and Montel (Neuropharm. 12:1073-80, 1973) established that NE-sensitive receptors located on terminal 5-HT nerve fibers regulate the release of 5-HT. More recently these receptors have been characterized as alpha$_2$ subtype. In view of this, the purpose of the present study was to evaluate NE and alpha$_2$ receptor regulation of K^+-induced 5-HT release in rat spinal cord synaptosomes and tissue slices.

Rat spinal cord synaptosomes or tissue slices (300 um) were loaded with [^{3}H]-5-HT (0.5 uM final concentration) and placed in a perfusion chamber and perfused with oxygenated Krebs Ringer buffer. Basal release was measured for 20 min in two minute fractions at which time K^+ (15 mM) was added to the perfusion medium for 20 min (Stauderman and Jones, Eur. J. Pharm. 120:107-10, 1986). When drugs were tested, they were present during the last 10 min of washing and throughout depolarization. The [^{3}H]-5-HT present in each fraction was expressed as a percentage of total releasable [^{3}H]-5-HT.

The addition of NE (1-1000 nM) reduced [^{3}H]-5-HT release in a concentration-dependent manner. Control K^+-stimulated release (1.58 + 0.39%) was reduced 18% by 1 nM NE and 54% by 100 nM NE. The alpha$_2$ receptor agonist clonidine also reduced [^{3}H]-5-HT release in a concentration-dependent manner with 100 nM reducing release from 2.09 + 0.4% to 1.0 + 0.3% (53% decrease, maximum effect). This effect was blocked by the alpha$_2$ antagonist yohimbine. In addition, basal release of [^{3}H]-5-HT was increased with the addition of yohimbine alone.

In order to determine if endogenous NE contributed to the regulation of 5-HT release, selective NE uptake inhibitors were included in the incubation medium during K^+-induced depolarization. Thus the endogenous NE released from tissue slices would be made available to regulate presynaptic receptors. Tomoxetine or desipramine selective NE uptake inhibitors, reduced the K^+-induced release of [^{3}H]-5-HT. Tomoxetine 1 uM reduced release 18% while 10 uM reduced release 40% and 10 uM desipramine, 20%. These results suggest that integration of NE and 5-HT terminal function in spinal cord could occur through NE influences on 5-HT presynaptic "heteroreceptor" systems.

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460.10 CHOLINERGIC STIMULATION OF SOMATOSTATIN SECRETION BY CEREBRAL CORTICAL CELLS IN VITRO. S. Halvonik* and R.J. Robbins. Section of Neuroendocrinology, Yale University School of Medicine, New Haven CT 06510

The coincidental loss of cholinergic and somatostatinergic (SS) markers from cortical regions of individuals with senile dementia of the Alzheimer's type led us to investigate possible interactions between these two systems. The observed short-lived inhibition of cortical electrical activity in response to acetylcholine (ACh; PNAS 82:6344) could be due to local release of an inhibitory transmitter such as SS. We hypothesized that ACh could stimulate SS release from cortical SS interneurons. Using primary monolayer cultures of rat cerebral cortical cells derived from 16 day old embryos, we examined the changes in the rate of release of immunoreactive SS (IRS) in response to addition of cholinergic agents. These cultures have been shown to synthesize and secrete both SS-14 and SS-28 (Endo 113:574). Cultures were used after 18-20 days in vitro (DIV). Medium was replaced by a Krebs-Ringer bicarbonate buffer, which itself was replaced every 20 min. IRS released per 20 min. incubation was measured by a sensitive and specific RIA. We found a rapid increase in IRS release in response to 1 uM ACh, which was 71% above baseline during the first incubation. This response returned to normal by 80 min despite the continued presence of ACh. Carbachol (C) also increased IRS release, with a much longer duration of action that seen with ACh. The lowest effective dose of C was 0.1 uM. Neither ACh nor C had an effect on cortical cells which were only 11 DIV. The effects of ACh were blocked by equimolar pirenzipine, QNX, and atropine. The action of C was blocked by QNX, atropine, and tetrodotoxin. The failure of AF64 pretreatment or of acute exposure to physostigmine to alter basal or stimulated IRS release suggested that endogenous cholinergic neurons, if present, were not altering IRS secretion. We conclude that SS neurons have the capacity to respond to cholinergic signals and that the loss of both markers in Alzheimer's disease may be due, in part, to this putative interaction. Supported by NIH grant AM36848 and ADRDA grant PRG 86-065.

460.11 OXOTREMORINE INHIBITS BETA-ADRENERGIC-RECEPTOR-MEDIATED EVENTS IN GLIA. V. Madelian, D.L. Martin, K. O'Connor* and W. Shain. Wadsworth Center Labs. and Res. New York State Dept. Health, Albany, NY 12201.

Astroglial cells release taurine in response to stimulation by beta-adrenergic agonists and other drugs and neurotransmitters. Studies of interactions among these agents have shown that taurine release is an integrated physiological response of the astroglial cell to the stimulation of multiple receptors. Since muscarinic cholinergic agents oppose the action of beta-adrenergic agonists in other systems, we investigated the effects of the muscarinic agonist oxotremorine (OXO) on isoproterenol (IPR)-stimulated taurine release and 3',5' cyclic adenosine monophosphate (cAMP) synthesis in LRM55 glial cells. For taurine release experiments, LRM55 cells were preloaded with [^{3}H]taurine for one hour and then superfused with Hepes-buffered Hank's solution supplemented with test drugs. Radioactivity was determined in samples taken at one min intervals. Application of OXO by itself at high concentrations (0.1 - 1 mM) weakly stimulated taurine release (less than 50% of the release observed with 100 nM IPR). However, OXO inhibited IPR-stimulated taurine release. Inhibition was weak (ca. 20%) when IPR and OXO were applied simultaneously for 3 min, but was almost complete when OXO was applied for 20 min before IPR. OXO also strongly inhibited the elevated steady-state release of taurine due to prolonged application of IPR. Since IPR-stimulated taurine release is mediated by cAMP, we also investigated the effects of OXO on IPR-stimulated cAMP synthesis. cAMP measurements were performed on cells preloaded with [^{3}H]-adenine and stimulated by adding drugs. Cells were solubilized in NaOH, proteins and non-cyclic nucleotides precipitated with $ZnSO_4$ and aliquots of the supernatant were injected onto an HPLC column to separate cAMP from other radiolabelled adenine derivatives. Fractions containing the cAMP were collected and radioactivity was measured by liquid scintillation counting. One min application of OXO at high concentrations (0.1 - 1 mM) had no effect on intracellular cAMP levels. However, OXO applied with IPR inhibited the IPR-induced cAMP accumulation by 20%. Pretreatment of cells with OXO resulted in a marked decrease of a subsequent IPR-induced response. This inhibition increased with the length of pretreatment and reached 50% by 1 hr. OXO also reduced cAMP levels in cells exposed to IPR for prolonged periods (30 min).

Thus, the inhibitory effect of OXO in IPR-induced taurine release is paralleled by its effect on IPR-induced cAMP accumulation suggesting that muscarinic regulation of beta-adrenergically induced release of taurine is mediated through regulation of cAMP levels. Supported by Grants NS21219 and AA07155.

460.12 HIBERNATION DEPENDENT INHIBITION OF HYPOTHALAMIC S-1 BINDING BY N-ACETYL SEROTONIN. P. R. Oeltgen*, D.L. Sparks, Lexington VA Med. Ctr. and Depts. of Pathology and Neurology, Univ. of KY Med. Center and Sanders-Brown Research Center on Aging. (SPON: D. T. Frazier). Dept. of Physio. Biophys., Univ. of KY Med. Center, Lexington, KY 40536.

Hibernation is the apparent suspension of physiologic time for an animal. Reductions of body temperature, heart rate, respiratory rate and oxygen consumption occur during hibernation and urine production and protein catabolism virtually cease. This remarkable circannual phenomenon is most aptly demonstrated in the rodential ground squirrel and woodchucks as well as the European hedgehog and the bat. The hypothalamus has been implicated in the onset of, maintenance of, and arousal from hibernation, especially in relation to temperature regulation. Serotonin is known to be important in a number of vegetative functions of the hypothalamus, and a relationship between hypothalamic function and hibernation has been proposed in a number of animal models, although exact mechanisms of interaction remain uncertain.

We have previously reported alterations in cholinergic-aminergic markers in the hypothalamus of hibernating ground squirrels. During hibernation the turnover and the binding of serotonin were reduced while choline acetyltransferase activity was enhanced.

During the course of these investigations, as previously reported, we found there was atrophy of the pineal gland during hibernation. The pineal gland produces melatonin via n-acetyl serotonin, from serotonin, but numerous studies indicate this does not occur during hibernation.

To determine if the pineal gland may have any effect on hypothalamic function, we determined the pharmacologic effect of pineal specific compounds on serotonergic binding in the hypothalamus from hibernating and non-hibernating ground squirrels. We now report state dependent novel pharmacology of serotonin binding in hypothalamus. Melatonin had little effect on (^{3}H)-spiperone binding and had no effect on (^{3}H)-5HT binding in hypothalamus during either hibernation or the active state. N-acetyl-serotonin (N-A-5HT) had no effect on (^{3}H)-spiperone binding during either state. On the other hand, N-A-5HT significantly inhibited (^{3}H)-5-HT binding (75%) in hypothalamus from hibernating ground squirrels, whereas little inhibition (20%) was found in non-hibernating animals. The possible relationship between hypothalamic and pineal function in hibernation will be discussed.

This work is supported in part by the U.S. Army Medical Research Acquisition Activity, Contract No. DAMD 17-87-c-7070, the Veterans Administration, the NIH (1-P01-AG05119), and by Alzheimer's Disease and Related Disorders Association, Inc. (II-86-078)

460.13 ADAPTIVE CHANGES IN HEART CHOLINERGIC-ADRENERGIC INTERACTIONS FOLLOWING 6-HYDROXYDOPAMINE TREATMENT. W.Y. Chen* and L.C. Wince. Dept. Zoological and Biomedical Sciences, and College of Osteopathic Medicine, Ohio University, Athens, OH 45701.

Heart function is controlled by a dual innervation by sympathetic (adrenergic) and parasympathetic (cholinergic) neurons. Changes in heart function are elicited by changes in the tonic activity of these two neuronal systems as they interact in a complex manner. Adrenergic (ADR) nerves release norepinephrine to increase heart contractile force, and cholinergic (CHOL) nerves release acetylcholine which, in heart ventricles, acts primarily to diminish ADR effects. The heart is known to adapt to changes in its neural input. Past research has focused largely upon adaptive phenomena in either the ADR or CHOL systems individually, but the relevance of such adaptive changes to ADR-CHOL interactions in the heart is unknown. The present study was performed to examine adaptive phenomena in CHOL-ADR interactions in the control of heart function. Sprague-Dawley rats (180-220 g) were injected i.v. with 6-hydroxydopamine (6-OHDA) in two doses of 50 mg/kg at 24 h intervals and sacrificed 1, 2, or 3 weeks following the initial dose. Papillary muscles were dissected from the left ventricle, suspended in a tissue bath containing oxygenated Krebs-Henseleit buffer, and electrically stimulated to elicit isometric contractions. The inotropic potency of the beta agonist isoproterenol (ISO) was studied in muscles from 6-OHDA treated and control rats. Supersensitivity to ISO was found at 1 and 2 weeks after 6-OHDA treatment as evidenced by 7.0 and 6.3-fold shifts, respectively, in the concentration- response curves. The ability of the muscarinic CHOL agonist carbachol (CARB) to attenuate the inotropic effects of ISO was also examined. In papillary muscles from control rats CARB (10 μM) reduced the inotropic effects of ISO as shown by a 2.2-fold shift of the concentration-response curve to the right. In contrast, CARB (10 μM) produced 9.1- and 9.4-fold shifts of the ISO concentration-response curve to the right at 1 and 2 weeks, respectively, after 6-OHDA treatment. We have also examined the ability of CARB to attenuate ISO-stimulated adenylate cyclase activity in ventricular homogenates. Preliminary results from these experiments indicate that CARB produces a greater shift to the right of the ISO concentration-response curve in homogenates from 6-OHDA treated animals at 2 weeks relative to controls. These results suggest that adaptive changes occur in the CHOL system after 6-OHDA treatment which increase the attenuation of the inotropic effects of ISO by CARB. Also, our preliminary data indicate that these findings may be related, in part, to an increased inhibition of ISO-stimulated adenylate cyclase activity by CARB. (Supported by Central Ohio Heart Chapter, AHA, Grant #C-86-41A and the Ohio Univ. Col. of Osteopathic Medicine.)

460.14 EFFECT OF SYSTEMIC KAINIC ACID ON REGIONAL TRH CONCENTRATIONS IN RAT CENTRAL NERVOUS SYSTEM. M.S. Kreider, B.L. Wolfinger* and A. Winokur. Dept. of Psychiatry, Univ. of Penna., Philadelphia, PA 19104

Several studies have suggested that TRH levels in the CNS are influenced by the level of CNS activation. Hibernation and estivation result in region-specific decreases in TRH concentrations. Repeated electroconvulsive shock and amygdala-kindling has been shown to result in elevations of TRH concentrations in limbic regions of the rat central nervous system. We investigated whether the production of limbic seizures by systemic administration of kainic acid would produce similar increases in TRH concentrations.

Male Sprague-Dawley rats (180-200 g) received injections of kainic acid, 12 mg/kg s.c, or saline. Rats were sacrificed 1, 2, 3, 4 or 7 days later. Brains were dissected into 10 regions, olfactory bulb, septum, corpus striatum, anterior cortex, brain stem, hypothalamus, posterior cortex, thalamus/midbrain, hippocampus and amygdala/piriform cortex. Each region was weighed, homogenized in phosphate buffered saline and extracted with methanol. Methanol extracts were dried, redissolved in 0.25% BSA/0.1 M sodium phosphate buffer, pH 7.6 and assayed for TRH content by radioimmunoassay.

Kainic acid administration resulted in very substantial increases in the concentration of TRH in anterior cortex, posterior cortex, hippocampus and amygdala/piriform cortex. Significant elevations were measured at all time points examined. Maximum increases of 300% for anterior cortex, 2700% for posterior cortex, 1600% for hippocampus and 300% fold for amygdala/piriform cortex were observed 3 days following administration of kainic acid. At 4 and 7 days, TRH concentrations of kainic acid treated rats were significantly higher than controls but were progressively lower compared to 3 day kainic acid treated rats. TRH concentrations in the remaining CNS regions were unaffected by kainic acid administration.

These results indicate that induction of limbic seizures by systemic administration of kainic acid is extremely effective in increasing TRH concentrations in limbic regions of the rat central nervous system. This may represent a useful model for investigations of the neurochemical systems involved in the regulation of TRH in the central nervous system.

460.15 GABAERGIC INNERVATION OF CORTICOTROPIN RELEASING FACTOR NEURONS IN THE RAT PARAVENTRICULAR NUCLEUS: AN ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL STUDY. J.A. Olschowka. Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, N.Y. 14642

The paraventricular nucleus (PVN) with its corticotropin releasing factor (CRF) neurons has been implicated in the control of biochemical, physiological and behavioral responses to environmental changes. However, the neural mechanisms underlying such adaptive responses have been difficult to unravel, and the anatomical circuitry remains largely undefined. Most recently, the CRF neurons of the PVN have been implicated in a number of clinical situations such as depression and anorexia. The high levels of plasma cortisol in these patients appears to be due to a defect in the regulation of CRF release which then results in hypersecretion. Accordingly, we have begun to analyze the various putative inhibitory inputs to these CRF neurons. This study reports the results of an anatomical analysis of GABA-containing terminals in relation to CRF neurons of the rat PVN.

The relationship of GABA terminals with CRF neurons was first examined by a double labeling immunocytochemical technique at the light microscopic level. Using an antiserum directed against glutamic acid decarboxylase (GAD, a gift from Dr. D. Schmeckel), the PVN of male Sprague Dawley rats was stained using an avidin-FITC label. Subsequently, CRF neurons in the same section were stained using avidin-rhodamine as a label. Within the fluorescence microscope, a dense plexus of GAD terminals was observed in close relation to many parvocellular CRF neurons, as well as in relation to unlabeled magnocellular neurons.

To study this putative interaction, a unique double labeling method was employed at the electron microscopic level. First, rats were perfused with an acrolein-parafomaldehyde mixture, vibratome sectioned and the tissue stained for GAD using a conventional avidin-biotin-peroxidase method. Following reaction with diaminobenzidine, the sections were washed and then incubated in an antiserum directed against rat CRF that had been previuosly labeled with fluorine (via carbodiimide coupling of nonadecafluorodecanoic acid). The sections were then washed, osmicated and embedded in Araldite 502. Unstained 30 nanometer sections were then viewed in a Zeiss EM902 analytical electron microscope. Numerous GAD-positive terminals were observed. In contrast to catecholamine and NPY terminals which more commonly form synaptic contacts on the distal portion of the dendritic tree, GAD terminals were observed forming numerous synaptic contacts on the soma of both parvocellular and magnocellular neuron, on their primary dendrites, as well as on the distal dendrites. Following localization of a GAD positive synaptic terminal, the postsynaptic element was then analyzed for the presence of the fluorine labeled anti-CRF using the EM902 and electron spectroscopic imaging. The presence of fluorine was detected in small, medium and large dendrites and in the soma of parvocellular neurons that received GAD synaptic contacts. This suggests that GABAergic terminals are located in large numbers and in a favorable positions on the CRF neuron in order to play a major inhibitory role in the regulation of CRF release. Supported in part by PHS grants NS20799 and NS24761.

461.1 DIFFERENTIAL EXPRESSION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR α-SUBUNIT mRNAs IN RAT BRAIN. E. Wada[1*], L.W. Swanson[1,2], J. Boulter[3*], K. Wada[3*], S. Heinemann[3] and J. Patrick[3]. [1]Neural Systems Laboratory., [2]Howard Hughes Medical Institute and [3]Molecular Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

We have identified three genes in the rat genome that code for nicotinic cholinergic receptors expressed in the brain. The genes encoding α-subunits of the nicotinic acetylcholine receptor gene family have been designated alpha1 (expressed in muscle) and alpha2, alpha3, and alpha4 (expressed in neurons). We have used *in situ* hybridization histochemistry to localize the sites of expression of these genes in the rat central nervous system. Because of the high homology in the protein coding regions of the cDNAs encoding these proteins we have used parts of the less conserved 3' untranslated sequences to make [^{35}S]RNA probes for *in situ* hybridization. Our initial results with these three probes demonstrate clear differential expression of alpha2, alpha3 and alpha4 in the medial habenula and the interpeduncular nucleus. Neuroanatomical data indicates that the medial habenula receives a cholinergic input from the basal forebrain by way of the stria medullaris and that cholinergic neurons in the medial habenula in turn project through the fasciculus retroflexus to form synapses in the interpeduncular nucleus. Our results indicate that the alpha3 and alpha4 genes are expressed at high levels within cells of the medial habenula while alpha2 transcripts are undetectable in this nucleus. In contrast, the alpha2 and alpha3 genes are expressed in the interpeduncular nucleus. These results clearly show that there is differential expression of the alpha2, alpha3, and alpha4 genes in cholinoceptive neurons of two distinct but interconnected nuclei in the rat brain. Recent experiments demonstrate that neurons in the medial habenula contain functional nicotinic cholinergic receptors (McCormick and Price, 1987, *J. of Neurosci.* 7:742). It seems likely that these receptors are encoded by the alpha3 and alpha4 genes. Likewise, nicotinic receptor mediated excitation has been observed in the interpeduncular nucleus (Brown, Pocherty, and Halliwell, 1983, *J. Physiol.* 341:655). These receptors may be encoded by the alpha2 and the alpha3 genes. These results suggest that the alpha2, alpha3 and alpha4 genes code for α-subunits of three different nicotinic receptor systems expressed in the brain.

461.2 IMMUNOHISTOCHEMICAL STAINING OF CHOLINE ACETYLTRANSFERASE IN CHICKEN MIDBRAIN. E.M. Sorenson, D. Parkinson and V.A. Chiappinelli. Dept. of Pharmacology, St. Louis Univ. Sch. Med., St. Louis, MO 63104 and Dept. of Cell Biology and Physiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

The chicken optic lobe contains high levels of choline acetyltransferase and acetylcholinesterase activities. It also contains high levels of α-bungarotoxin and nicotine binding sites compared to other tissues. The optic lobe has therefore been used to characterize and purify putative central nicotinic receptors by several laboratories. Monoclonal antibodies have also been used to localize putative nicotinic receptors with label found in the nucleus spiriform laterale and its processes in the tectum (Swanson, L.W., et al., PNAS USA 80:4532, 1983).

To further define cholinergic transmission in the chicken midbrain, we have localized acetylcholine-containing neurons and their processes. Although acetylcholinesterase positive cells have been localized in optic tectum, the biosynthetic enzyme choline acetyltransferase (ChAT) is considered a more reliable marker for acetylcholine-containing neurons. A polyclonal antiserum (Johnson, C.D. and Epstein, M.L., J. Neurochem. 46:968, 1986) to chicken ChAT was therefore used to localize the ChAT-containing neurons in chicken midbrain.

An adult chicken brain was immersion fixed with 4% paraformaldehyde in .01 M sodium phosphate, pH 7.4. Parasagittal and horizontal slices, 60 μ thick, were cut on a microtome and pretreated with 1% hydrogen peroxide to destroy endogenous peroxidase. Free floating slices were incubated overnight with primary antiserum in dilutions of 1:3000 to 1:5000. An avidin-biotin-horseradish peroxidase staining procedure using diaminobenzidine and cobalt enhancement was used to visualize the primary antiserum.

Several areas of the chicken midbrain were stained for choline acetyltransferase. Darkly stained cell bodies were seen in tectal layer IIi and the nuclei isthmi parvocellularis, semiluminaris and spiriform laterale. Stained fibers appear throughout the tectum as well as in the nuclei isthmi magnocellularis, intercollicularis and corpus geniculatum laterale ventrale. Fibers lateral to the labelled cells in the spiriform laterale are also stained. The cells stained in tectal layer IIi are radial neurons and their processes can be seen running in the tectum.

The localization of choline acetyltransferase containing cells and fibers further defines areas of cholinergic transmission in the chicken midbrain. This, together with other data, will help in the localization of putative nicotinic receptors and in distinguishing their possible functions as they are better characterized within different areas of the chicken midbrain.

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461.3 NEURONS FROM THE PERIBRACHIAL REGION PROJECT TO THE MEDIAL PRF: WGA-HRP AND FLUORESCENCE STUDY IN THE CAT. P.J. Shiromani, A. Berkowitz*, D.M. Armstrong, P.M. Groves, and J.C. Gillin. Depts. Psychiat & Neurosci (DMA), SDVAMC & UCSD, La Jolla, CA 92093.

The medial pontine reticular formation (mPRF), which lies between the abducens and trigeminal nuclei, is implicated in REM sleep (REMS) generation. Evidence from both extracellular and intracellular studies indicate that mPRF neurons depolarize before REMS onset and maintain a tonic level of depolarization throughout REMS. The early depolarization of some mPRF neurons, therefore, may recruit other reticular neurons so that when a sufficient number are depolarized and a threshold of excitation is reached then REMS is generated. We hypothesize that acetylcholine is the neurotransmitter responsible for initiating the early depolarization in some mPRF neurons because (i) cholinergic agonists are the only class of compounds that rapidly trigger REMS, (ii) cholinergic antagonists block this effect and (iii) some mPRF neurons increase discharge before and in conjunction with carbachol-induced REMS. However, these studies indicate that the mPRF is cholinoceptive. Since there are no cholinergic cells in the mPRF, a cholinergic input is required to depolarize the mPRF neurons instrumental to REMS generation. In this study we demonstrate that the neurons from the peribrachial region, where a major collection of cholinergic cells are found, project to the mPRF REMS area.

Two cats were deeply anesthetized and stereotaxic microinjections of either Fluorogold (2%, 0.1 ul) or WGA-HRP (1%, 0.1 ul) were made into the mPRF. Either 48 or 120 hrs later the cats were administered an overdose of Nembutal and perfused intracardially with PBS followed by 2% formaldehyde-glutaraldehyde PBS. The brains were soaked in 30% sucrose and then 40 um thick sections obtained using a freezing microtome. The sections were processed for HRP using TMB as the chromagen. Fluorogold sections were examined using wide-band uv excitation.

We observed a tight collection of HRP and fluorescent cells in the peribrachial region. The labelling was noted mainly in the rostral (A3-A0) portions of the peribrachial region, in an area where we have shown the presence of cholinergic neurons. Estimates based on gross examination of the area suggest that less than 20% of peribrachial neurons were labelled. The labelling was noted mainly in bipolar, tripolar and multipolar cells with bipolar cells being the most numerous. No labelling was seen in the lateral dorsal tegmental area where cholinergic cells have also been observed.

These preliminary results suggest a cholinergic input from the pedunculopontine region to the mPRF. However, it remains to be determined that cholinergic cells project to the mPRF.

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461.4 QUANTITATIVE AUTORADIOGRAPHIC ANALYSES OF SELECTIVE MUSCARINIC RECEPTOR AGONIST BINDING WITHIN REGIONS OF RAT BRAIN. W. Hoss., B.R. Ellerbrock*, M.A. Price* AND W.S. Messer, Jr. Department of Medicinal Chemistry, College of Pharmacy, University of Toledo, Toledo OH 43606.

The binding of four muscarinic receptor agonists to regions of rat brain were examined through quantitative autoradiographic techniques. Oxotremorine, arecoline, pilocarpine and bethanechol were chosen based on their different relative potencies and efficacies in muscarinic second messenger systems. Overall, the order of potency for inhibition of [^{3}H]-l-quinuclidinyl benzilate binding to rat brain slices was oxotremorine > pilocarpine ≥ arecoline >> bethanechol. Regional assays of agonist potency indicated that all agonists were more selective for brainstem and thalamic regions than for hippocampal and cortical regions. Thus all of the agonists were M_2-selective. It appears that differences in binding do not explain the differences in abilities of the agonists to activate second messenger systems.

Of the four agonists examined, pilocarpine displayed the highest selectivity for M_1 receptors in that IC_{50} values for pilocarpine were only three-fold higher in the hippocampal and striatal regions (e.g., CA3: 40.6 ± 9.4 μM) than in thalamic and brainstem regions (e.g., posteroventral thalamus: 14.9 ± 6.2 μM). Oxotremorine was eight-fold more potent in the brainstem and thalamus, while arecoline and bethanechol were 19- and 100-fold more selective for brainstem and thalamic receptors.

Scatchard analyses revealed heterogeneous binding profiles for some agonists within single brain regions, suggesting that multiple receptor sites exist even within regions of predominately M_1 or M_2 receptors. For example, arecoline displayed curved Scatchard plots within the external layers of the cerebral cortex, layer CA1 of the hippocampus (predominately M_1 subtype) and the posteroventral thalamus (predominately M_2 subtype). The ability for agonists to recognize multiple sites within a single region may reflect the ability to recognize receptors coupled or uncoupled to second messenger systems (e.g., GTPase).

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461.5 DISTRIBUTION OF MUSCARINIC CHOLINERGIC RECEPTORS IN THE DORSAL VAGAL COMPLEX AND OTHER SELECTED NUCLEI IN THE HUMAN MEDULLA. T.M. Hyde, M. Gibbs and S.J. Peroutka. Depts. of Neurology and Pharmacology, Stanford University Medical Center, Stanford, CA 94305.

The dorsal vagal complex (area postrema, nucleus of the solitary tract, and dorsal motor nucleus of the vagus) has been implicated in a wide variety of neurovisceral interactions. A neurochemical analysis of the dorsal vagal complex may allow a greater understanding of the function of this region in the modulation of visceral activity. (e.g the mechanism of action of antiemetics). Studies in the rat (Wamsley, J.K. et al. J. Neurosci. 1:176, 1981), cow and squirrel monkey (Pedigo, N.W. et al. Brain Res. Bull. 14:1, 1985), and human (Cortes, R. et al. Neurosci. 12:1003, 1984) have demonstrated the presence of muscarinic cholinergic receptors in the dorsal vagal complex. We have extended this analysis to the subnuclear distribution of these receptors in order to define their role in the neurovisceral axis.

Muscarinic cholinergic receptors were localized in human medulla by quantitative autoradiography, using the radioligand ^{3}H-quinuclidinyl benzilate. Receptor densities were highest in the hypoglossal nucleus. The second highest density was found in the medial region of the nucleus of the solitary tract (NTS). Moderately high numbers of receptors were present in the dorsal motor nucleus of the vagus, the dorsal NTS, subpostremal NTS, lateral NTS. and ventral NTS. Intermediate densities were present in the dorsal and medial accessory nuclei of the inferior olive and the spinal trigeminal nucleus pars interpolaris. Low densities were found in the area postrema, principle nucleus of the inferior olive, gracile nucleus, cuneate nucleus, and the tract of the NTS. The concentration of these receptors in the medial NTS, which in non-human primates is the principle site of abdominal visceral input to the NTS, suggests that they are part of the neural network subserving gastrointestinal modulation. In particular, the antiemetic action of scopolamine may be explained by its effects on these receptors.

461.6 THE IN VIVO DISTRIBUTION OF [^{11}C]SCOPOLAMINE IN HUMAN BRAIN DETERMINED BY POSITRON EMISSION TOMOGRAPHY. K.A. Frey, R.A. Koeppe*, D.M. Jewett*, G.K. Mulholland*, R.D. Hichwa*, D.E. Kuhl and B.W. Agranoff. Cyclotron/P.E.T. Facility, University of Michigan, Ann Arbor, MI 48109.

Previous work from this laboratory has evaluated the use of [^{3}H]scopolamine for in vivo measurement of muscarinic cholinergic receptors in the rat brain (Frey et al., J. Neurosci. 5:421, 1985). In the present work, the in vivo determination of muscarinic receptor binding is extended to the study of human brain with the use of [^{11}C]scopolamine and positron emission tomography (PET).

[N-methyl-^{11}C]scopolamine was synthesized from norscopolamine by reductive methylation with [^{11}C]formaldehyde. The product was purified by HPLC and formulated in sterile saline containing 10% ethanol for intravenous injection. Four neurologically and medically normal volunteers between the ages of 22 and 60 were studied. Experiments were initiated with the intravenous injection of 20 to 40 mCi of [^{11}C]scopolamine (specific activity 580 to 1000 Ci/mmol). Serial PET scans of the head were obtained over the subsequent 120 min using a TCC PCT-4600A tomograph in the high resolution mode. Arterial blood samples were withdrawn from a radial artery catheter at intervals for determination of the plasma scopolamine activity curve. After centrifugation, samples of the plasma were applied to liquid chromatographic columns (SEP-PAK C_{18}) for separation of scopolamine from labeled metabolites prior to activity measurement in a gamma counter.

Results of the four studies were qualitatively similar. During the first minute significant tracer concentrations were observed in major vascular structures (carotid arteries, venous sinuses). Within the first few minutes following injection, brain parenchymal tracer concentrations increased rapidly, with greater uptake by gray than white matter structures. At longer times following injection, a characteristic distribution was observed which is similar to that determined previously in animal studies. The structures with highest uptake included cerebral cortex, basal ganglia, thalamus and the pons. The cerebellar hemispheres, although initially similiar to cerebral cortex in activity, were considerably less labeled in the later images. These results confirm the previous animal experiments and indicate that factors in addition to the density of muscarinic receptors as measured in vitro contribute to the distribution of [^{11}C]scopolamine in vivo. Subsequent kinetic analysis of the tracer distribution may distinguish the binding process from tracer uptake and non-specific distribution.

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461.7 TRACER KINETIC ANALYSIS OF [^{11}C]SCOPOLAMINE BINDING IN HUMAN BRAIN. R.A. Koeppe*, K.A. Frey, D.E. Kuhl and B.W. Agranoff (SPON: A.M. Heacock). Cyclotron/P.E.T. Facility, University of Michigan, Ann Arbor, MI 48109.

The kinetics of in vivo scopolamine binding to muscarinic receptors have been modeled, and temporal counting studies performed in rats using [^{3}H]scopolamine (Frey et al. PNAS 82:6711, 1985). We present here initial results from in vivo human studies of [^{11}C]scopolamine kinetics in brain using positron emission tomography (PET).

Four normal male volunteers were scanned following injection of 20-40 mCi of [^{11}C]scopolamine. A dynamic sequence of 18 PET scans covering two hours was acquired (5x1 min; 4x2.5 min; 3x5 min; 3x10 min; 3x20 min). Blood samples were drawn from the radial artery as rapidly as possible for 2 min and then at progressively longer intervals throughout the remainder of the study. Plasma scopolamine was counted following separation from labeled metabolites. The kinetics were analyzed assuming a three-compartment model: a plasma compartment; a tissue compartment including both free and non-specifically bound tracer (assumed to equilibrate rapidly); and a tissue compartment representing specifically bound tracer. Three rate parameters were estimated using non-linear least squares analysis. Two parameters describe exchange of tracer between plasma and brain, defining the transport or uptake rate (blood flow x extraction) and the tracer's apparent distribution volume in tissue (excluding specifically bound scopolamine). The third parameter describes the product of the ligand/receptor association rate and the available receptor density. Dissociation was considered to be negligible during the course of the study.

The transport rate of scopolamine in cortex, deep gray structures and cerebellum, ranged from 0.07 to 0.10 ml/g/min, while white matter, rates were 0.04-0.05, indicating extractions of ~10 and 20% respectively. The binding rate constant averaged 0.21 min^{-1} (0.1-0.35) in cortical and subcortical gray matter, while binding in cerebellum was ten-fold lower, ranging from 0.01 to 0.025. Estimates of the distribution volume ranged from 3 to 8 ml/g, however, as predicted by simulation studies (Koeppe et al. J Nucl Med 27:P936, 1986), this parameter estimate has very high uncertainty. Estimates of the binding rate and distribution volume were very sensitive to input function shape, stressing the importance of accurate correction for labeled metabolites in arterial samples.

PET images at two hours show clear distinctions in tracer distribution between cortex and cerebellum, but concentrations differ only by a factor of two due to significant non-specific binding. However, kinetic analysis reveals the the ten-fold or greater difference in binding between cortex and cerebellum. Supported by NIH Grants #NS15655 and #NS24896.

461.8 IDENTIFICATION OF SUBSTANCE P IN CELL BODIES OF NASAL AFFERENTS IN THE TRIGEMINAL GANGLION OF THE CAT. G.E. Lucier and R. Egizii*. Department of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 4N1 Canada.

The ethmoidal nerve innervates the anterior portion of he nasal mucosa and constitutes the afferent limb of several upper respiratory tract protective reflexes. Recent research in this laboratory has traced the central projections of the ethmoidal nerve to the trigeminal ganglion and various sub-nuclei of the spinal trigeminal nucleus (Lucier and Egizii, J. Comp. Neurol., 247:123-132, 1986). In addition, single-unit recording from teased fibers in the ethmoidal nerve and brainstem recording from second order neurons has revealed that ethmoidal receptors respond to tactile and noxious stimuli (Lucier and Egizii, Neurosci. Abstr., 10:236.1, 1984). The purpose of this study was to identify the percentage of cell bodies within the trigeminal ganglion which not only had projection from the ethmoidal nerve but also exhibited the presence of endogenous Substance P (SP), a neurotransmitter believed to play a role in nociceptive transmission.

Adult cats were anaesthetized with a halothane/oxygen mixture. The ethmoidal nerve was exposed by complete enucleation of the eyeball. The peripheral cut end was then placed on a small piece of laboratory film and pure crystals of horseradish peroxidase (HRP) (Sigma Type VI) were applied to the nerve and left for one hour, after which time the area was sealed with low-melting point wax, to prevent leakage. Following a survival period of 72 hours, the animals were perfused with 4% paraformaldehyde. The trigeminal ganglia were post-fixed and transferred into 30% sucrose overnight at 4C. Frozen 40 um longitudinal sections were cut and reacted for HRP reaction product using 1% cobalt chloride, 1% nickel ammonium sulphate and 3,3'-diaminobenzidine (DAB). They were then further processed for immunohistochemical demonstration of endogenous SP using a bi-specific rat monoclonal antibody against SP. The distributions of HRP only (presence of black granular reaction product within the cell bodies), SP only (uniform tan-colored cellular staining) or double-labelled neurons (HRP + SP) were plotted throughout the ganglia.

HRP reaction product was present only in the ipsilateral ganglia, while SP immunoreactivity was present in both ipsilateral and contralateral ganglia. Approximately 25% of cells labelled with HRP also contained SP. These results support our previous electrophysiological data indicating that between 17-30% of ethmoidal afferents are nociceptive. (Supported by MRC).

461.9 THREE-DIMENSIONAL LOCALIZATION OF ^{125}I-NEUROTENSIN BINDING SITES IN THE RAT MESENCEPHALON USING DIGITIZED IMAGE PROCESSINGS.
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We have recently shown that high densities of neurotensin (NT) binding sites are present in both rat substantia nigra (SN) and ventral tegmental area (VTA). In those brain regions several lines of evidence suggest that NT binding sites are located on dopaminergic cell bodies and dendrites.

The aim of the present work was to develop a computerized approach for three-dimensional representation of the localization of NT binding sites in both SN and VTA.

We considered the entire structures in a three-dimensional space using 80 serial mesencephalic sections obtained from one rat brain, incubated with monoiodo Tyr3 NT and treated for radioautographic analysis on LKB film as previously described (Sarrieau et al; Brain Res. 348; 375, 1985).

A video camera coupled with an IBAS image analyzer was used to sequentially digitize the series of autoradiograms as well as the structure outlines after respective alignment. The labeled regions were discriminated with a densitometric threshold. We developped a three-dimensional reconstruction algorithm (based on voxel representation) which enabled us to visualize the inside of the reconstructed objects.

Because of several unavoidable alterations of the slice's shape mainly during sectioning and mounting, the irregularities observed in the reconstructed 3-D shape for each brain were corrected with an original non-linear lowpass three-dimensional filter.

An opaque and three-dimensional picture with different shadings was then obtained at various orientations which simultaneously represent with one color both VTA and SN outlines, and with another one the labeled areas.

NT binding sites are observed in the zona incerta and their density increases when the SN pars compacta appears. Caudally, both dorsal and ventral labeled areas of the SN pars compacta describe a ring around the SN pars reticulata. The latter is less intensively labeled and shows a great number of labeled stripes which probably correspond to labeled dendrites. On the contrary, labeling in the VTA is homogeneous along the rostro-caudal extension of the structure. The 3D labeled areas do not exactly correspond to the outlines of the defined structures, which may be explained by the fact that not all cells in both VTA and SN are labeled with ^{125}I-NT.

The 3D representation gives more synthetical morphological informations than the mere use of bidimensional picture of brain slices; this may be a helpful new approach to study the extreme complexity of the spatial organization of the brain.

461.10 DISTRIBUTION OF CHOLECYSTOKININ RECEPTORS IN PRIMATE MEDIAL TEMPORAL AREA. M.F. Kritzer, R.B. Innis and P.S. Goldman-Rakic. Sec. Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT. 06510.

Cholecystokinin (CCK) binding sites were localized in the hippocampus, amygdala, and medial temporal cortices of macaque monkeys using in vitro receptor autoradiography. Binding sites were labeled with ^{3}H-CCK-8 and ^{125}I-CCK-33 and non-specific binding was assessed in the presence of 1 uM CCK-8. Comparison of autoradiograms with Nissl stained sections allowed precise correlation of autoradiographic grain distribution with cytoarchitecture.

In the hippocampal formation a single dense band of label was observed over the granule cell layer and adjacent few millimeters of the molecular layer of the dentate gyrus. This heavy band of label stood out sharply from the very low density binding of both the polymorph and remaining molecular layers. Binding was similarly concentrated in the presubiculum, particularly over the densely packed pyramids, clearly distinguishing this region from the adjacent subiculum in which binding just exceeded background levels. Moderate to light label was observed in the hilus and stratum pyramidale of CA3 while remaining hippocampal layers showed minimal specific binding. CCK binding in the amygdala varied among nuclear subdivisions. The lateral, basomedial, endopiriform, and cortical nuclei showed dense CCK binding, while labeling was notably sparse in the central, medial, basolateral, and accessory basal nuclei. Variations in CCK binding in the medial temporal cortex showed close correspondence to cytoarchitectonic divisions. Thus, in entorhinal cortex binding was concentrated in layers III, IV, and V, while, binding in area TH was more evenly distributed among layers. Area TF was distinguished from other regions by the emergence of a bilaminar pattern of labeling in which binding sites were concentrated in layers II and IV.

The variation in CCK binding throughout the medial temporal lobe suggests that in this region CCK exerts selective rather than diffuse influence on cellular elements. This influence may include modulation of afferents arising from prefrontal cortex, which are anatomically coincident with CCK binding in the lateral amygdaloid nucleus, the external granule cell layer of the presubiculum, and deep layers of entorhinal cortex (Goldman-Rakic et al., 1984, Neurosci. 12: 719; Aggleton et al., 1980, Brain Res. 190: 347). These findings may also bear on recent evidence of CCK depletion in temporal lobe structures in schizophrenics (Ferrier et al., 1983 Life Sci. 33: 475) whose predominant 'negative' symptoms of flattened affect and lack of initiative resemble frontal lobe dysfunction. Supported by MH38546 and NS22807.

461.11 TRH RECEPTORS IN THE CNS OF THE AFRICAN LUNGFISH. A.M. Pack*, S.B. Caine*, A. Winokur and A.P. Fishman*. Depts. of Pharmacology, Psychiatry and Cardiovascular Pulmonary Division of the Dept. of Medicine, Univ. of Penna., Philadelphia, PA 19104.

The African lungfish (Protopterus) contains high concentrations of thyrotropin releasing hormone (TRH) in the central nervous system, with concentrations in the telencephalon and diencephalon ranging from 5-10 fold higher than in comparable regions of rat brain. TRH produces potent effects on respiration in several species, and has also been shown to exert changes in behavioral arousal when micro-injected into discrete regions of the CNS. The lungfish is the first air breathing species, and also undergoes a naturalistic change in behavioral state known as aestivation. To further investigate the lungfish as a model for studying the effects of TRH on respiration and behavioral arousal in the CNS, we examined the localization, quantification, and characterization of TRH receptors in the CNS of the lungfish.

Saturation binding studies utilizing the high-affinity ligand [3H]-MeTRH revealed a single receptor with a Kd of 3.2 nM, a Hill coefficent of 1.0, and a Bmax of 177.4 fmol/mg protein in whole sections from lungfish telencephalon. This concentration of TRH receptors was 3-4 fold higher than that found in whole sections from any region of rat brain, with a Kd similar to that found in mammalian species. Inhibition binding studies revealed that MeTRH is approximately ten times more potent at inhibiting the binding of [3H]-MeTRH than TRH, with IC50s of 1.3 nM for MeTRH and 15.8 nM for TRH. This agrees well with the potency of these analogs in inhibiting binding of [3H]-MeTRH in the rat CNS. Quantitative autoradiography revealed a heterogeneous distribution of TRH receptors in the CNS of the lungfish, with highest receptor density in regions of the telencephalon and olfactory bulb. High receptor density was also found in regions of the diencephalon and dorsal horn of the spinal cord, and moderate to low receptor density was found throughout these regions as well as in the medulla. Taken together, these findings suggest that the CNS of the lungfish contains high concentrations of a specific TRH receptor with pharmacological characteristics similar to that of the mammalian TRH receptor. The localization and quantification of TRH receptors in the CNS of the lungfish should facilitate studies of this species as a model for the CNS actions of TRH.

461.12 BIDIRECTIONAL TRANSPORT OF ANGIOTENSIN II BINDING SITES IN THE VAGUS NERVE. D.I. Diz and C.M. Ferrario. Department of Brain and Vascular Research, Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44106.

We have previously shown that specific angiotensin II (Ang II) binding sites are present in the canine nodose ganglion (NG) and peripheral vagus nerve (VN). Unilateral removal of the NG results in loss of Ang II binding sites in the ipsilateral nucleus tractus solitarii and the dorsal motor nucleus of the vagus. These findings suggest an association of Ang II binding sites with both afferent and efferent vagal systems and are consistent with the known actions of the peptide to influence the baroreflex. To investigate possible transport of Ang II binding sites in the VN, quantitative in vitro receptor autoradiography was used to visualize binding after double ligation of the peripheral process of the cervical VN in 8 dogs. Under halothane anesthesia, one ligature (LIG1) was tied 0.2 - 0.5 cm from the NG and the second ligature (LIG2) was tied on the same nerve 1.0-1.5 cm from the NG. Twenty-four hours later, the dogs were perfused with phosphate buffered saline containing 0.04% formalin. The untouched and ligated VN were removed and frozen. Ang II binding was determined by incubating 14-20 µm sections in sodium phosphate buffer containing dithiothreitol, EDTA, NaCl, $MgCl_2$, and 0.4% bovine serum albumin plus 0.01-4.0 nM ^{125}I-Ang II. Nonspecific binding was assessed by addition of 1 µM unlabeled Ang II. Analysis of autoradiographic images confirmed the presence of specific, high affinity Ang II binding sites in the cell body region of the NG (K_a = 0.66 ± 0.08 nM) and the trunk of the VN (K_a = 0.83 ± 0.26 nM). After ligation, Ang II binding sites (K_a = 0.51 ± 0.08 nM) accumulated at LIG1 (on the side nearest the NG), revealing anterograde transport. Retrograde transport of binding sites also occurred since accumulations of a similar affinity site (K_a = 0.52 ± 0.08 nM) were seen distal to LIG2. These data reveal the existence of a mechanism for the bidirectional axonal transport of Ang II binding sites in the cervical portion of the canine VN. Further studies are needed to determine whether Ang II binding site transport is present in afferent or efferent fibers, or a combination of both, and whether it occurs in association with bound peptide. These data provide new information on the site(s) and mechanism of action of Ang II on cardiovascular and possibly other autonomic reflexes. (Supported in part by NHLBI Grant HL-6835 and the Reinberger Foundation).

461.13 MATCHING ANGIOTENSIN WITH ITS RECEPTORS IN RAT BRAIN: IMMUNOHISTOCHEMISTRY AND LIGAND BINDING. R. Wallace Lind, Andrew M. Allen*, Fred A.O. Mendelsohn*, and Detlev Ganten*. The Salk Institute, La Jolla, CA 92037; The University of Melbourne, Australia; The Univerisity of Heidelberg, Germany.

Neuroanatomical approaches to understanding angiotensin in the brain address two fundamental questions: Where is the peptide produced and where does it act. To answer the first question, immunohistochemistry has been used to identify neuronal cell groups that contain angiotensin immunoreactivity. It is assumed that such cells synthesize the antigen and ship it out over efferent fiber pathways for use as a chemical messenger. Towards answering the second question, binding of labeled angiotensin (or any of its high-affinity analogues) has identified presumptive sites of action. It is assumed that at locations where binding occurs there are membrane-bound receptors that detect the presence of the ligand diffusing across a synapse. If angiotensin does indeed act according to these prevailing notions about the behavior of neurotransmitters in the brain, and if our techniques possess adequate fidelity and sensitivity, the location of immunohistochemically identified cell groups and fiber systems should form a complement to the distribution of binding sites. To put it another way, one would expect to find evidence of angiotensin receptors in the places where angiotensin-containing neurons send their axons.

The results of the present work establish that such a complementary relationship does exist in the rat brain. We have found at least thirty distinct locations where angiotensin-immunoreactive terminal fields overlap concentrations of binding sites. In some instances, for example in the bed nucleus of the stria terminal and in the paraventricular of the hypothalamus, the co-distribution of terminals and receptors is concordant at the subnuclear level. In other cases, for example in the ventral subdivision of the lateral geniculate nucleus, the two techniques provide evidence for a hitherto unknown site of action of angiotensin. And in other cases, for example in the projection from the tufted and mitral cells of the olfactory bulb to layer Ia of piriform cortex, improvements in immunohistochemical staining have identified an angiotensin-containing pathway that was suggested by earlier binding work.

In conclusion, the overwhelming agreement between these two converging lines of evidence gives us a list of likely sites of action. The next steps are to identify the parent cell groups giving rise to the various terminal fields, and to clarify the efferent traffic resulting from the activation of the several receptor pools. Eventually, such an understanding of morphology will form the groundwork for an elucidation of the full range of functions subserved by angiotensin in the brain.

461.14 PRESENCE OF RELAXIN-LIKE PEPTIDES IN RAT BRAIN. Charles Bakhit, Helen Kado-Fong*, Derf Lewis*, Geoff Tregear* and Bernard Malfroy*. Pharmacological Sciences, Genentech, Inc., South San Francisco, California 94080, and Howard Florey Institute, Melbourne, Australia.

Relaxin is a polypeptide hormone found in blood and reproductive tissues of a wide variety of nonmammalian and mammalian species ranging from sharks to humans. Relaxin is found in decidua, placenta and ovarian corpora lutea as well as testes, seminal plasma and prostate glands.

The physiological role of relaxin is poorly understood. Relaxin has effects on uterine activity, cervical dilatation during late pregnancy, and sperm motility. More recently, relaxin has been shown to modulate oxytocin and vasopressin release from hypothalamic nerve terminals in vitro.

The only known source of relaxin in the adult rat is the corpora lutea of the female. We hypothesized that relaxin may be produced by other organs, specifically the brain.

Extracts from ovaries and brain from nonpregnant and pregnant (day 20) female rats, and also testes and brain from male rats were prepared and analyzed by Western blot. Relaxin-like immunoreactivity was detected in brains of the pregnant and nonpregnant rats but only in the ovaries of pregnant rats. In the male rat, relaxin like immunoreactivity was found in the brain and testes.

Northern blot hybridization analysis of polyadenylated mRNA from brains and ovaries of pregnant and nonpregnant rats using a probe encoding rat relaxin showed two bands one at 0.6 kb and a more predominant band at 1.8 kb. Analysis of mRNA from specific brain regions of female rats showed the presence of mRNA encoding rat relaxin in cortex, hippocampus, hypothalamus, striatum and cerebellum.

The above data suggest that relaxin-like peptides are not only produced by the rat ovary, but also by the brain. To our knowledge this is the first demonstration of the presence of relaxin in rat brain.

NEURAL PLASTICITY IN ADULT ANIMALS IV

462.1 NEUROGENESIS IN THE EIGHTH NERVE OF AN ADULT TELEOST: SOURCE OF THE NEW NEURONS. J. C. Presson and A. N. Popper Department of Anatomy and Cell Biology, Georgetown University, Washington D.C. 20007

The number of neurons in the saccular portion of the eighth nerve increases with fish size in adults of the teleost species Astronotus ocellatus (Popper and Hoxter, Hearing Res, 1984). The source and location of the new neurons, however, are not known. To ascertain the source of the new cells, adult oscars, 5 cm in standard length, were injected with tritiated thymidine. Each fish recieved a total of four injections of 5 microCuries/gbw. The injections were separated in time by 1 week. Following the last injection the animals survived for 4-6 weeks. The eighth nerves were embedded in plastic (JB-4), sectioned at 4 microns and processed in a standard fashion for autoradiography.

Following this injection schedule no mature eighth nerve neurons were labeled with thymidine. However, examination of the sections revealed small clusters of cells that are clearly different in morphology from either eighth nerve neurons or glial cells. A few of the cells in these clusters were labeled with tritiated thymidine. The cells in the clusters are about 5 microns in diameter, show dark dark, granular nuclear staining with Nissl stain, and have no detectable surrounding cytoplasm. Surrounding the clusters are cells with larger, pale staining nuclei, and little or no cytoplasm. Surrounding these are cells that look like small eighth nerve neurons, but with no apparent procesess. Based upon these observation we suggests that the clusters of small cells as the precursors to new eighth nerve neurons, and the surrounding cells as differentiating eighth nerve neurons.

In a single saccular eighth nerve there are 2 or 3 such clusters. The clusters are connected by a thin string of what appear to be similar types of cells. Thin strings of these cells also connect the saccular clusters to similar clusters in the lagenar and utricular portions of the eighth nerve. The clusters vary in size, suggesting that the number of eighth nerve neurons produced may vary accross the epithelium. Experiments designed to verify these cells as eighth nerve precursors are in progress. (Supported by NIH)

462.2 ESTROGEN-INDUCED ALTERATIONS IN SYNAPTIC MORPHOLOGY IN THE MIDBRAIN CENTRAL GRAY. R.S. Cohen, S.K. Chung, and D.W. Pfaff. Dept. of Anatomy, Univ. of Illinois at Chicago, Chicago, IL 60612 and Dept. of Neurobiology and Behavior, The Rockefeller Univ., New York, NY 10021.

Axons of ventromedial hypothalamic (VMH) neurons have been previously shown to terminate in the midbrain central gray (MCG) (Chung et al., 1984, 1986). Since VMH synapses in this region may be involved in the mediation of estrogen-induced lordosis behavior, we examined the effect of estrogen on the morphology of synapses in the MCG. Ovariectomized (ovx) adult female rats were given daily subcutaneous injections of estradiol benzoate (10 μg) (n=3) or the vehicle control (n=3) and after 20 days of injection, only the estrogen-treated (ovx+E) rats showed the lordosis response. MCG tissue was fixed and processed according to standard electron microscopy procedures. A quantitative analysis of MCG tissue demonstrated morphological changes in several synaptic parameters with estrogen treatment. The micrographs were coded and randomly arranged before the analysis, so that the observer was blind to the experimental condition. There was an increase in the mean number of dense-cored vesicles (dcvs) per synapse in the MCG of the ovx+E group (1.49 ± 0.04) compared to the ovx group (0.46 ± 0.04) ($p=0.0001$). The MCG of the ovx+E group also contained a higher percentage (60%) of synapses showing dcvs than the ovx group (28%) ($p=0.0001$). There was a higher mean length of postsynaptic densities (PSDs) per synapse in the MCG of the ovx+E group (39.78 ± 0.86 nm) than in the controls (28 ± 0.81nm) ($p=0.0001$). In addition, the percentage of PSDs containing one or more perforations was higher in the ovx+E group (15%) than in the controls (10%) ($p<0.05$). There was also a greater percentage of synapses showing positive curvature in the ovx+E group (62%) compared to the ovx animals (36%) ($p<0.05$). A higher overall number of synapses was seen in the MCG of the ovx+E group (3.64 ± 0.12) compared to the controls (2.73 ± 0.12) ($p=0.0001$). No significant differences were seen in the percentage of synapses showing subjunctional bodies beneath PSDs in the two groups.

The dcvs are similar in size and appearance to those that increased in number in the VMH of ovx+E animals (Cohen and Pfaff, 1981), suggesting that they may be transported from the soma of VMH neurons to synaptic endings in the MCG. These dcvs may contain a neurotransmitter, neuromodulator, or a trophic factor (Chung, 1986). Such transmitters or peptides may induce morphological changes in postsynaptic structures which are related to the effects of estrogen on lordosis. Supported by NIH grants HD 05751 and NS 15889.

462.3 SYNAPTIC REORGANIZATION IN THE MEDIAL AMYGDALOID NUCLEUS AFTER LESION OF THE ACCESSORY OLFACTORY BULB OF ADULT RAT.
M.Ichikawa. Dept. of Anatomy & Embryology, Tokyo Metropolitan Inst. for Neurosciences. Fuchu. Tokyo. Japan.

Synaptic reorganization in the accessory olfactory pathway of rat whose function had been well known was examined for the first step toward understaning the correlation between lesion-induced synaptic reorganization and recovery of function after injury.

Time-course of the loss and reappearence of synapses was investigated in the medial amygdaloid nucleus(MAN) following lesions of the accessory olfactory bulb(AOB). A quantitative electron microscopic analysis of the molecular layer was performed after the AOB lesion. Synaptic density was reduced to less than half of the control density at 4 or 8 days after the lesion. Thereafter,synaptic density increased more than 80 % of control density at 64 days after lesion. The recovery in number of synapses following the lesion suggests the possibility of synaptic reorganization by remaining afferent fibers.

Rearrangement of terminations from the bed nucleus of stria terminalis(BST) was examined in the MAN at 2 months following the lesion of the AOB using an electron microscopy and degeneration study. At 2 days following a BST lesion, the number of degenerating synapses was 0.7 ± 0.1 per unit area in the molecular layer. At 2 months after AOB lesion, the degenerating synapses completely disappeared from MAN. The BST was then lesioned at 2 monthe after AOB lesion and the degenerating synapses were counted in MAN at 2 days following the BST lesion. The number of degenrating synapses were 3.3 ±0.6 per unit area in the molecular layer. The number of these degenerating synapses increased significantly in the molecular layer after AOB lesion.

Rearrangement of intra-amygdaloid connections after the AOB lesion was examined further in MAN by use of PHA-L immunohistchemistry. PHA-L was injected in the posteromedial region of amygdala(posteromedial amygdaloid nucleus and amygdala-hippocampal transitional area). The labeling axons were observed only in the cellular part of MAN. After the AOB lesion, the labeling axons were found not only in the cellular part but also in the molecular layer.

All these results show that the synaptic reorganization of axons following denervation of the AOB fibers occured in MAN. To elucidate the correlation between the lesion-induced synaptic reorganization and the functional recovery after injury, the study of changes of the accessory olfactory function after the AOB lesion is necessory.

462.4 SELECTIVE LONG-TERM POTENTIATION IN THE PYRIFORM CORTEX: ROLE OF GABAERGIC MECHANISMS. C. A. Gramlich and J. S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

The potential evoked in the pyriform cortex (PC) by electrical stimulation of the olfactory bulb (OB) has two components: period 1, which reflects excitatory input from the OB and feedforward excitation within the PC, and period 2, which is contemporaneous with and may partially reflect GABA-mediated inhibition in the PC. Diazepam (4 mg/kg), which facilitates GABAergic transmission, was given intravenously to male Long-Evans rats implanted with chronic electrodes to examine the role of GABA in potentials evoked in the PC by OB stimulation, and in the potentiation of period 2 that appears in the PC after high-frequency stimulation of the OB (Soc. Neurosci. Abstr. 10:76 1984).

In naive rats, diazepam (DZP) significantly increased period 2 duration without affecting input from the OB (as reflected by period 1 amplitude). Following this initial test, half of the rats received repeated high-frequency stimulation of the OB (15 trains of ten 400-microA pulses at 100 pulses/sec), while the remaining rats served as low-frequency controls (150 pulses at 0.5 pulses/sec). This stimulation was repeated four times at three day intervals. High-frequency stimulation produced an increase in the duration of period 2 and in the amplitude of a late component that intruded into period 2. These effects lasted at least three days. No change was seen in period 1. Changes in control animals were negligible.

Following potentiation, the effects of DZP on the expression of potentiation were examined. DZP increased period 2 duration as well as the amplitude of the late component referred to above.

In this study the effects of DZP paralleled rather closely those of potentiation on the PC evoked potential. This finding suggests that potentiation of period 2 in the PC is mediated at least in part by increased activity at GABAergic synapses, an interpretation that is supported by evidence of enhanced functional inhibition in the PC during period 2 (Soc. Neurosci. Abstr. 11:780, 1985).

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462.5 LOCUS COERULEUS LESIONS PREVENT VIBRISSECTOMY-INDUCED METABOLIC PLASTICITY IN ADULT RAT BARREL CORTEX. B.E. Levin, R.L. Craik and P.J. Hand. VA Med. Ctr., E. Orange, NJ 07019, N.J. Med. Sch., Newark, NJ 07103, Beaver College, Glenside, PA 19038 and Univ. of Penn. Sch. of Med. and Vet. Med., Phila., PA 19104.

The barrel cortex of the adult rat is known to undergo plastic changes related to chronic removal of afferent input from the facial vibrissae. Stimulation of facial vibrissae normally produces increased glucose utilization (LCGU) in the corresponding barrels (lamina IV) and their associated columns in laminae I-VIa of the contralateral rat first somatosensory cortex (SmI) as assessed autoradiographically by uptake of ^{14}C 2-deoxyglucose (2-dg). The current studies were designed to evaluate the relationship between noradrenergic innervation of the SmI cortex and its response to chronic deafferentation. Bilateral surgical removal of all but the C3 facial vibrissae was performed in adult male Sprague-Dawley rats. After 2mo, the areal extent of the metabolic representation of the SmI barrels and columns corresponding to the spared C3 vibrissae was increased 39% (p=0.001) and 31% (p=0.003), respectively. No change in LCGU occurred in these enlarged barrels and columns as compared to non-surgical controls. In other rats with intact facial vibrissae, unilateral locus coeruleus (LC) lesions with 6-hydroxydopamine depleted ipsilateral cortical norepinephrine (NE) levels by 90%. Two months later, the areal extent of the metabolic representation of the C3 barrels and columns was increased 11% (p=0.05) and 21% (p=0.05) ipsilateral to the LC lesions vs. the intact side. No changes in LCGU occurred in the C3 representation. In additional rats, bilateral vibrissectomy with sparing of C3 vibrissae was paired with unilateral LC lesions causing 90% ipsilateral cortical NE depletion. Here the areal extent of the spared C3 metabolic barrels and columns ipsilateral to LC lesions failed to enlarge comparably to those on the contralateral side with intact LC (20% difference; p=0.05). Again, there were no differences in LCGU in the C3 representation. Therefore, chronic cortical NE depletion increased the areal extent of 2-dg uptake in the barrel and column of the SmI cortex corresponding to a stimulated vibrissa without affecting overall LCGU in rats with intact facial vibrissae. However, similar NE depletion blocked the plastic enlargement of the metabolic representation of the spared vibrissa in the SmI cortex after chronic vibrissectomy. This suggests that NE has opposing effects on cerebral metabolism in intact and chronically deafferented SmI cortex.

462.6 ADULT FOLIATE PAPILLAE ADD NEW TASTE BUDS AFTER THE PERIPHERAL FIELD OF THE CHORDA TYMPANI NERVE IS CONDENSED. D.R. Riddle*, S.E. Hughes and B. Oakley. Washington. Univ., St. Louis, MO. and Dept. of Biol., Univ. of Michigan, Ann Arbor, MI 48109.

In mongolian gerbils, Meriones unguiculatus, we condensed the projection of the chorda tympani (CT) fibers in the tongue by suturing the proximal stump of the chorda-lingual nerve to the most posterior of its three branches. We wished to determine if directing additional CT fibers to the rear of the tongue would result in the formation of supernumerary foliate or fungiform taste buds. In addition, since taste buds supported by the CT degenerate after it is sectioned but are reformed after appropriate innervation is restored, the experiment allowed us to assay the ability of misdirected CT fibers to reinnervate the tip of the tongue.

Only 30% of the fungiform taste buds were reformed on the anterior portion of the tongue (vs >95% in controls), suggesting the CT fibers were largely constrained to the area of their entry into the tongue and had only limited ability to reinnervate the tip. Although the density of regenerating CT fibers was, at least initially, higher than normal in the middle and posterior fungiform field, no more taste buds than normal were found in this region (25+7, N=15 vs 28+4, N=15 in controls, mean+SD). These and related experiments performed by the authors suggest that the nature of the gustatory epithelium, not the level of innervation, limits the number of fungiform taste buds. Such limits could be due to the nature of the stem cells or the structure of the fungiform papilla.

After the peripheral projection of the CT was condensed, 165+23 taste buds were found in the foliate papilla (N=12). This is significantly more than the 130+18 found in control papillae (N=13, p<.001, Mann-Whitney, one-tailed). There was no change in the mean number of slits in the papilla (9.5+1.5 vs 9.2+1.1 in controls). The distribution of the foliate taste buds suggests new taste buds were added to each slit. In normal animals the IXth nerve can support all of the foliate taste buds (136+27, N=4), while the CT alone will support only 26+12 (N=4). After the IXth nerve was removed from experimental animals, several months after the CT had been condensed, the condensed nerve alone supported only 19+7 foliate taste buds (N=3). Thus, it appears that the IXth nerve helped maintain the 30+ extra taste buds since, without the IXth nerve, the condensed CT maintained no more foliate taste buds than a normal CT. We are now extending the evaluation of the seperate roles of the CT and IXth nerves in the formation and maintenance of the added foliate taste buds. This is the first demonstration that extra taste buds are formed in adult papillae in response to hyperinnervation.

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462.7 PLASTICITY OF VASOTOCIN-LIKE IMMUNOREACTIVE SYSTEMS IN THE CANARY BRAIN. E.R. De Kloet, Th.A.M. Voorhuis, J.A.M. Van Eekelen, J.Z. Kiss and D. De Wied. Rudolf Magnus Institute for Pharmacology, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands. (SPON: ENA)

Vasotocin (VT) immunoreactive cell bodies and fibers were demonstrated by immunocytochemistry in the brain of the canary (Serinus canarius). Birds were perfused by a two step fixation method: first a low pH and then a high pH of the fixative. Coronal vibratome sections (50 µm) were processed and incubated with antiserum. For visualization of the antigen-antibody reaction we used the avidin- biotin peroxidase complex. Three diencephalic regions contain VT cells: first, the anterior preoptic nucleus and the periventricular magnocellular nucleus (PVM). The PVM is the equivalent of the paraventricular nucleus in the rat; second, the lateral parts of the hypothalamus, c.q. the supraoptic nucleus in the rat; third, the basal septal area and the nucleus stria terminalis. Axons leaving the PVM together with axons from cells in the lateral hypothalamus form the hypothalamo-hypophyseal tract. The medial posterior hypothalamic nucleus c.f. the rat ventromedial nucleus shows dense innervation. Extrahypothalamic areas receive fibers from the PVM. There is a strong innervation of lateral and medial septum, lateral habenula, the substantia grisea centralis, the locus coeruleus, the raphe nuclei, the nucleus tractus solitarius and lateral medulla. These nuclei are thought to be involved in mediation of limbic and autonomic function. VT fibers are present in structures related to sensory functions: e.g. the optic tectum and the nucleus ovoidalis. Finally, VT fibers innervate centers which have been implicated in vocal control: nucleus robustus archistriatalis and nucleus intercollicularis. Immunoreactive cells and fibers displayed plasticity due to gonadal influences. The male canary shows a denser VT innervation of the lateral septum and has more VT immunostained neurons in the dorsal diencephalon than the female canary. Castration of male canaries resulted in a decrease of VT immunoreactivity. Chronic testosterone replacement of the castrated males via silastic implants restored the intensity of the original immunostained fibers and cells. In the female canary testosterone also increased fiber and cell immunostaining in these brain regions.

462.8 **THE ULTRASTRUCTURAL DISTRIBUTION OF INNERVATION WITHIN THE RAT PINEAL GLAND ARISING FROM THE TWO SUPERIOR CERVICAL GANGLIA.** R.E.Zigmond, J.C.Henderson and R.H.Loring. Dept. of Pharmacology, Harvard Med. School, Boston, MA 02115

Serotonin N-acetyltransferase (NAT) in the rat pineal gland exhibits a circadian rhythm, with activity being highest at night. This rhythm is believed to result from a nocturnal increase in the firing of the sympathetic neurons that innervate the gland. NAT activity is abolished by a bilateral lesion of the preganglionic cervical sympathetic trunks (CST) or the postganglionic internal carotid nerves (ICN). During the first night after a unilateral lesion of either the CST or the ICN, NAT activity is reduced by 75%. By the second night after such an ICN lesion, NAT activity recovers to normal, but after a unilateral CST lesion, enzyme activity shows no recovery for up to 5 months (J. Neurosci. 5: 142, 1985). We have hypothesized that the lack of recovery in the latter group is due to decentralized sympathetic neurons, which, though electrophysiologically silent, are capable of inhibiting the synaptic efficacy of the contralateral, active sympathetic neurons by taking up transmitter released by the latter ("heteroneuronal uptake"). When pineal glands are fixed with mixed aldehydes, most varicosities contain small synaptic vesicles with dense cores during the day but not at night, suggesting that the dense cored vesicles are associated with neurons with low firing rates. In control animals and in animals in which one or both CST were cut, 14%, 52% or 85%, respectively of the varicosities examined at night were "tagged" with dense cored vesicles. These values correspond well to the expected percentages of inactive neurons innervating the pineals of these animals. We have used this morphological marker to study the distribution of "active" and "inactive" varicosities. Varicosities occurred in clusters, and, in animals with one CST cut, these clusters often contained both tagged and untagged varicosities. The average distance between an untagged varicosity and the nearest tagged varicosity was 2.2 um, while the distance between an untagged varicosity and the nearest pinealocyte was 2.5 um. These data suggest that varicosities arising from the two superior cervical ganglia are in close proximity in the pineal gland, and thus support our hypothesis that heteroneuronal uptake may regulate synaptic efficacy. (NS12751)

462.9 EFFECTS OF BRAIN GROWTH AND GLUCOCORTICOIDS ON BEHAVIORAL FLEXIBILITY IN THE RADIAL MAZE. T.L. Thomas and L.D. Devenport. Department of Psychology, University of Oklahoma, Norman, OK 73019.

Bilateral adrenalectomy (ADX) produces an increase in brain volume and weight that is arrested but not reversed by later corticosterone (Cort) replacement (1). In young rats this growth takes the form of increased cell division, including myelinogenesis and possibly glial proliferation (2). The growth effects of ADX are site-specific and independent of type I receptor density, affecting instead neocortex, thalamus, and certain fiber tracts (3). Thus, much is known about the structural changes produced by ADX, but nothing about the functional consequences of the CNS growth. This study begins to address the question.

Female SD rats (35d) were subjected to ADX and allowed to mature for 40 d. During this time half of all the subjects were maintained on a 7 mg/kg/24 hr s.c. dose of Cort. This dose has previously been shown to maintain normal brain size in ADX rats (2). The other animals were given a 2 mg/kg/24 hr dose that was sufficient to maintain health, but insufficient to arrest ADX-induced brain growth. During this time, the rats were trained to run for food reward to four arms of an 8-arm radial maze, after which the hormone conditions were changed for half the animals, yielding groups designated in terms of dose as 2-2, 7-7, 2-7, 7-2. This 2 x 2 design provided a control for current hormone and the experience of hormone change apart from the long-term influence on brain size, which was our major interest.

Three days after hormone change, the rats were presented with a reversal problem in the maze. Their response to this problem was the target behavior of the study and required the animals to give up running to the formerly baited arms and to explore the maze until discovering the new contingency. The results revealed a strong interaction between brain size and current hormone. Both big-brained groups (2-2 and 2-7) exhibited large increases in run time and ancillary exploration at reversal, but only the 2-2 group continued to increase beyond the first day. The 7-7 group exhibited little change in behavior at reversal or on later days.

While current hormone level can influence an animal's responsiveness to environmental change, the degree of influence is significantly dependent upon brain size. The reversal procedure induces some degree of exploratory behaviors in all groups except one (7-7). This may indicate that the smaller brains are deficient in information-gathering as well as uniquely responsive to Cort.

1. Devenport & Devenport. (1986). Exp. Neurol., 90, 44.
2. Meyer. (1983). Exp. Neurol., 82, 432.
3. Thomas & Devenport. (1985). Soc. Neurosci. 11, 899.

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462.10 THE NMDA ANTAGONIST AP5 BLOCKS A COMPONENT OF THE POSTSYNAPTIC RESPONSE TO THETA BURST STIMULATION AND PREVENTS LTP INDUCTION. J. Larson* and G. Lynch (SPON:M.-L. Kean). Center for the Neurobiol. of Learning and Memory, Univ. of Cal., Irvine, CA, 92717.

Short bursts of high frequency stimulation (4 pulses, 100 Hz) produce maximal long-term potentiation (LTP) at Schaffer/commissural synapses on CA1 neurons in hippocampal slices when the bursts are spaced 200 ms apart. A single burst to one set of fibers does not induce LTP but "primes" the post-synaptic neurons such that the depolarization produced by a burst to a second input 200 ms later is much larger and LTP is induced. The present experiments show that part of the response enhancement produced by priming is mediated by N-methyl-D-aspartate (NMDA) receptors and that the NMDA component is necessary for the development of synaptic potentiation.

Theta patterned burst stimulation to induce LTP consisted of pairs of bursts to the priming and test inputs separated by 200 ms; the pairs were given ten times at 5 sec. intervals. Dendritic field EPSPs were recorded in response to single-pulse stimulation of the test input; the response to a burst was quantified as the area of negativity evoked by the 4 pulses in the burst.

2-amino-5-phosphonovalerate (AP5-100uM) did not significantly alter responses to moderate intensity single pulses. However, it did completely prevent LTP induction by burst stimulation (EPSP potentiation in AP5: 0.4%+2.8%; after washout: 35.7%+6.7%).

AP5 produced a small depression of the response to the priming burst (8.8%+3.0%) but a larger and more consistent reduction of the response enhancement caused by priming (enhancement relative to an unprimed burst was 34.7%+9.2% in AP5 and 52.7% +10.7% after washout). Moreover, AP5 blocked the response potentiation that developed across repeated primed bursts.

In summary, AP5 completely blocked the short and long-term forms of synaptic potentiation produced by patterned burst stimulation. The response enhancement observed in primed bursts that induce LTP appears to have two components, one of which is mediated by NMDA receptors. The NMDA receptor is known to be voltage-sensitive; it seems likely that the non-NMDA component of the primed burst response provides sufficient depolarization to allow activation of the NMDA response and this response is then responsible for producing synaptic potentiation. Since the short bursts used in these experiments are similar to naturally occurring discharge patterns of hippocampal cells and the optimal interburst interval corresponds to the period of the theta rhythm, the results suggest links between these two aspects of hippocampal physiology and a receptor type that promotes synaptic plasticity. (Supported by AFOSR 86-0099 and ONR N00014-86-K-0333.)

462.11 ENLARGEMENT OF SYNAPSES IN THE STRIATUM FOLLOWING PARTIAL DEAFFERENTATION. S. Chen and D.E. Hillman. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York 10016

Reactive synaptogenesis has been demonstrated in many regions of the brain in response to a reduction of afferent inputs after environmental insults. Modification in the size, shape, and location of synapses as well as formation of new synapses are important means to restore functional integrity between remaining afferents and their target neurons. Previously, we showed that mature Purkinje cells had a rapid, marked synaptic reorganization following lesioning to granule cells or their parallel fibers. Here we study the reactive changes in the striatum following undercutting of the frontal cortex in adult rats. The animals were perfused with fixative at 2 to 40 days after surgery. There was a rapid and marked plasticity in the principal caudate neurons. Degenerating boutons were numerous but few vacant spines were seen within 2 days after surgery and only a few were still present after 2 weeks. Phagocytosis was almost complete by 40 days with occasional dense bodies and myelin remnants. Enlarged synapses occurred by 2 days and were evident throughout all the period. Dense core vesicles were not found in these enlarged presynaptic boutons indicating that dopaminergic fibers may not be significantly involved in synaptic compensation. There was an accompanying increase in the frequency of multiple synaptic contacts on enlarged boutons. These were from the remaining cerebral cortical connections. The complexity in the shape and size of synapses was analyzed by three-dimensional reconstruction. The enlarged synapses had increased synaptic contact area and a marked irregular boundary with deep indentations. Frequently, perforations occurred as small holes. The contact surface undulated between the spine and bouton generating an enlarged synaptic contact area and intercellular contact zone. The larger synapses had single and double protrusions of the bouton that invaginated the spine head and were capped by synaptic specializations. Quantitative analysis of synaptic profile lengths revealed that the average size of the synaptic area increased at 2 days following lesioning and remained large but was less complex at 40 days. Accompanying this was a decrease in the number of synapses to the principal striatal neurons. [Supported by USPH grants NS13742 & HD20349.]

462.12 INCREASED STRIATAL PROTEIN PHOSPHORYLATION IN THE RAT MODEL OF PERSISTENT DYSKINESIA INDUCED BY IMINODIPROPIONITRILE (IDPN). Jean Lud Cadet, Mirdu Birmani* and Harish Pant. Columbia University, New York, NY 10032 and NIAAA, Rockville, MD 20852

IDPN causes a set of behavioral abnormalities consisting of hyperactivity, random circling, and spasmodic neck dyskinesias. These behaviors can be attenuated by noradrenergic antagonism. Moreover, the brain of IDPN-treated rats show a significant increase in prazosin binding which is accompanied by similar changes in NE-stimulated phosphotidyl inositol (PI) turnover in the striata. It was thus proposed that the syndrome may be associated with changes in calcium and protein kinase C (PKC)-dependent protein phosphorylation in the brains of IDPN-treated animals (Cadet et al. Soc. Neurosci. Abst. 1986). The present study was carried out to test this idea.

Rats were treated with IDPN (100 mg/kg) until the development of the dyskinetic syndrome (7 days). Subsequently, controls and treated animals were killed at various time intervals and their striata were rapidly dissected out and placed in a homogenizing buffer made up of 20 mM Hepes, 10 mM BME, .05% leupeptin, pH 7.2. Phosphorylation studies were started after preincubation with either calcium and/or cAMP, calmodulin, or phosphotidyl serine (PS) by the addition of 32p-ATP. The reaction was stopped after 3 min. The striata of dyskinetic animals showed significant increases in the PS-dependent phosphorylation of a 66-68 kd and of 100 kd protein. The 66-68 kd protein may correspond to the neurofilament protein of similar size which is known to be affected by IDPN treatment. The 100 kd protein may be related to the stress glycoprotein which is induceable by calcium ionophore, glucose deprivation, and stress (Welch et al. JBC 258,7102, 1983). The stress glycoprotein is found on both the plasma membrane and the golgi apparatus. The manner by which IDPN causes these changes and their relation to other biochemical abnormalities reported in IDPN-treated animals remain to be determined.

In any case, these results are in agreement with the idea that phosphorylation of neurofilament proteins may play a role in neurodegeneration and in IDPN neurotoxic effects. These data also suggest that studies of phosphoproteins in human dyskinetic disorders may be a fruitful area of research.

462.13 PYRIFORM CORTEX KINDLING AND VASOPRESSIN LEVELS IN SPONTANEOUSLY HYPERTENSIVE RATS. R.S. Greenwood, R. Meeker, and J.N. Hayward. Dept of Neurol. and Neurobiology Prg., UNC Sch. of Med., Chapel Hill, NC 27514

There is growing evidence that vasopressin may serve as a neuromodulator or neurotransmitter in the nervous system and that it may have a role in the pathogenesis of nervous system disorders such as kindling. Homozygous Brattleboro rats, deficient in vasopressin, have significantly altered rates of kindling (Gillis and Cain, 1983) and repeated intracerebroventricular vasopressin injections have been reported to induce epileptiform activity (Kasting et al 1980). We have previously shown that with amygdala stimulation spontaneously hypertensive rats (SHR) are more rapidly kindled than control, parent strain, Wistar-Kyoto normal rats (WKYN) (Greenwood et al, 1985). The SHR have been reported to have higher circulating levels of vasopressin and urinary excretion of vasopressin than WKYN. In the present study we sought to compare the SHR and WKYN rats' kindling rates with pyriform kindling. We also compared the resting hematocrit (Hct), osmolality (Osmol), and vasopressin level (VP) of SHR and WKYN rats to determine if these might correlate with any kindling differences between SHR and WKYN.

Anesthetized SHR and WKYN rats were stereotaxically implanted with bipolar stimulating electrodes placed in the right pyriform cortex. Daily kindling stimulation (1 sec train, 60 cps, 400 uA) was continued until the animal reached 3 consecutive stage 5 seizures. A chronic indwelling catheter was used to obtain resting blood samples for Hct, Osmol, and VP (Greenwood et.al. 1986).

Kindled seizures were behaviorally similar in SHR and WKYN rats. SHR reached stage 5 seizures after significantly fewer after-discharge (AD) than WKYN rats. In addition, AD duration was significantly longer in SHR. Resting vasopressin levels of SHR (5.6 $\pm$ 1.1 pg/ml) were 40% higher than in WKYN rats (3.9 $\pm$ 0.5 pg/ml). Serum osmolalities were also higher in SHR.

We conclude that, as with amygdala kindling, SHR kindle more rapidly than WKYN rats with pyriform cortex kindling. Vasopressin levels are higher in SHR. Together these results and previous studies suggest that the alternations in kindling rate and vasopressin may have a common biological origin. Supported in part by USPHS NS00724 AND Javits Award NS13411

462.14 NEUROMUSCULAR PLASTICITY FOLLOWING SUBTOTAL DISUSE. M.A. Fahim., USC Andrus Gerontology Center and Biology Dept., Los Angeles, CA 90089-0191.

The neuromuscular junction (NMJ) undergoes remodelling from early development through senescence but the rapidity with which this occurs and the role of altered activity are relatively unexplored. Muscle activity appears to regulate both pre- and post-synaptic characteristics (Reviews: Robbins, N., Trends in Neurosci. 120, 1980; Grinnel, A.D. & Herrera, A.A. Prog. Neurobiol. 17, 1981).

One of the best documented forms of neuromuscular disuse, which last 2-3 weeks, produces about 90% disuse and does not entail use of drugs or nerve injury, is obtained by limb immobilization (Fischbach, G.D. and Robbins, N., J. Physiol. 201, 1969). Since this procedure procedure changes in synaptic transmission within 3-5 days, sprouting and longitudinal distortion of nerve terminals, flattening of primary grooves within 5 days, we sought ultrastructural evidence of synapse remodelling under the same conditions using transmission electron microscopy. We describe here a rapid ultrastructural response to subtotal neuromuscular disuse. The response occurs within 5 days and involves both pre- and post-synaptic elements.

The effects of 5 days immobilization the ultrastructure of the male Sprague-Dawley rat NMJs were studied both qualitatively and quantitatively. The Soleus muscle was immobilized by unilateral pinning of ankle and knee joints at right angles for 5 days. Immobilized muscles were then compared to contralateral muscles and normal litter mates. Muscle fiber diameters of immobilized animals were found to be significantly reduced by 31% and 29.5% when compared to those contralateral and control muscles respectively. Evidence of degeneration and regeneration in the same NMJs occurred in all three groups but were significantly greater in the disused muscles. These results reveal, in the adult nervous system, considerable and rapid ultrastructural remodelling induced by only a brief period of partial disuse.

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462.15 CHRONIC EFFECTS OF ADULT INFRAORBITAL (IO) NERVE TRANSECTION UPON RESPONSE PROPERTIES OF CELLS IN TRIGEMINAL (V) SUBNUCLEUS INTERPOLARIS (SpVi). B.G. Klein, L.A. Morse*, M. Barcia*, R.W. Rhoades and M.F. Jacquin. Dept. of Neurosci., New York Coll. of Osteo. Med., Old Westbury, N.Y. 11568 and Dept. of Anat., UMDNJ- Sch. of Osteo. Med. and R.W. Johnson Med. Sch., Piscataway, N.J. 08854.

Transection of the IO nerve in neonatal or adult rats results in anatomical and functional reorganization of the primary afferent pathway from whiskerpad to V brainstem nuclei. Alterations of the structure and function of trigeminal second-order neurons in adults have also been demonstrated following neonatal IO nerve damage. In this study, we used glass microelectrodes, orthodromic and antidromic electrical stimulation, and receptive field mapping to describe the response properties of SpVi cells more than 60 days after adult IO nerve transection. 132 cells were characterized in SpVi ipsilateral to the lesion (105 local circuit, LC; 22 thalamic projecting, TH; 5 cerebellar projecting, CB) and compared with 95 SpVi cells from normal adult rats (74 LC, 11 TH, 9 CB, 1 TH + CB). All units were examined by the same experimenter (B.G.K.). Characteristics of these neurons are summarized in the table below. Recording sites were histologically verified.

ALL CELLS	NORMAL	NERVE CUT
IO receptive field	91.6%	62.9%
No field	0.0%	17.4%
Interdivisional convergence (excluding whisker convergence in TH cells)	1.1%	9.1%
Cervical convergence	0.0%	2.3%
CELLS WITH IO RECEPTIVE FIELDS		
Whisker responsive, light touch	87.4%	63.8%
Guard hair responsive, light touch	5.7%	21.7%
Skin responsive, light touch	1.1%	8.4%
Pinch or deep pressure	6.9%	10.8%
Split receptive fields	0.0%	27.7%
Spontaneously active	18.4%	28.9%
CELLS WITH MYSTACIAL WHISKER FIELDS		
Guard hair or skin convergence	3.9%	28.3%
LC neurons responding to >1 whisker	5.2%	37.8%

Thus, chronic effects of adult IO nerve transection include changes in 1) numbers of SpVi cells activated from the IO region of the face, 2) receptor modalities driving SpVi cells, 3) intra- and 4) inter-receptor modality convergence, 5) inter-divisional convergence, 6) cervical convergence and 7) receptive field continuity (eg. split fields). The respective roles of primary and central afferents to SpVi in the functional alterations noted above, await further clarification. Support DE07734, DE07662, the AOA.

462.16 THE CAPACITY FOR NEUROGENESIS AND AXON RECONNECTION PERSISTS IN THE OLD ADULT NERVOUS SYSTEM. R.M. Costanzo and E.E. Morrison. Dept. of Physiology, Medical College of Virginia, Richmond, VA 23298.

It has been shown that neurogenesis, cell replacement and axon reconnection occurs in the olfactory system of young animals. In addition, newly developed axons have been shown to reestablish functional connections with cortical cells in the olfactory bulb (Br. Res. 361:258-266, 1985).

In the present study we examined the capacity of the newly developed axons to reconnect with the mature brain (olfactory bulb) in old adult hamsters. A new method was developed for making complete transection of sensory fibers projecting to the olfactory bulb. HRP tracing techniques were used to examine the extent of developing axon projections originating from olfactory receptor cells. We observed axon projections growing from receptor neurons across the transection site to the olfactory bulb. Further examination of the olfactory bulb revealed that axon terminals formed glomerular-like structures and made synaptic connections with second order cells in the olfactory bulb.

The results demonstrate that the mammalian central nervous system of old adult animals maintains the capacity for neurogenesis and axon reconnection. These findings may have important implications for replacement and repair of damaged pathways in the adult CNS.

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462.17 CONTRIBUTION OF INHIBITION TO THE COOPERATIVITY THRESHOLD FOR LONG-TERM POTENTIATION. R.M. Douglas and A. Dempster*, Dept. Physiology and Biophysics, Dalhousie University, Halifax, N.S. Canada.

Long-term potentiation (LTP) of perforant path synapses is only induced when a relatively large number of the excitatory synapses are coactive (McNaughton et al, Brain Res., 1978). This cooperativity requirement for LTP can be met by using moderate stimulation intensities to activate a high percentage of perforant path axons. In possibly related phenomena, LTP is blocked by activation of an inhibitory pathway to the dentate area (Douglas et al, Brain Res., 1982), and addition of picrotoxin, a GABA antagonist, greatly enhances the amount of LTP observed in vitro (Wigstrom & Gustafsson, Nature, 1983). To examine in vivo the role of inhibition in creating the cooperativity threshold, and to localize the responsible inhibitory synapses, iontophoresis of bicuculline was used to block GABAergic inhibition at depths in the dentate gyrus corresponding to different postsynaptic sites.

Rats were anesthetized with urethane, and stimulation intensities adjusted to just below that needed to evoke population spikes. After first demonstrating that high frequency trains (500 Hz for 20-40ms) at these intensities were inadequate for producing LTP of the field EPSP, subsequent identical trains were given while iontophoresing bicuculline methiodide (5-40 nA for 4 min.). By itself bicuculline had no effect on the field EPSP, but did facilitate the population spike when applied near the cell bodies. Ejection in the molecular layer had no effect on the population spike, indicating that diffusion away from the recording site was not significant.

Although LTP was sometimes observed after trains with ejections in the hilus, the most sensitive area was the dentate molecular layer. Frequently, in the presence of bicuculline, LTP could be obtained in the perforant path synaptic zone and not in the cell body level. The lowest currents (5nA) were adequate when the perforant path intensity was very close to the LTP threshold, as evidenced by a small amount of LTP to the initial control train. The threshold was not completely abolished however, as much lower intensity trains failed to produce LTP even with the higher ejection currents. When a series of trains was used to produce a large and saturated LTP, addition of bicuculline to subsequent trains had no effect, thereby demonstrating that the bicuculline did not increase the maximum amount the synapses could potentiate.

The results support the idea that the cooperativity threshold for LTP is set partially by inhibitory mechanisms, and strongly suggest that the critical inhibitory synapses lie not on the cell bodies but on dendrites in the molecular layer.

462.18 THE ROLE OF THE LOCUS CERULEUS IN RECOVERY OF FUNCTION FROM SENSORIMOTOR CORTEX INJURY. K.A. Krobert, M.G. Boyeson, and P. J. Scherer. Dept. of Rehab Med. Univ. Wis. Med. Sch., Madison, WI 53706

Following the observation that infusions of norepinephrine into the cerebellum contralateral to a sensorimotor cortex injury (SMCX) facilitated recovery from hemiparesis (Boyeson, et al. Neurosci Abstr, 1986) efforts were directed to the role of the locus ceruleus (LC) in the recovery process. In the first series of experiments the interval between a right LC lesion and right SMCX suction ablation was manipulated as follows: simultaneous LC and SMCX injury, LC lesion one week prior to SMCX, and LC lesion 2 weeks prior to SMCX injury. In all groups, NE infused into contralateral cerebellum permanently facilitated recovery at 24 hours post SMCX injury. Additionally,a remarkable NE-dependent jump in functional recovery occurred shortly after infusion in the 2 groups where a week or 2 weeks separated the LC and SMCX injuries. This was not observed in the simultaneous LC-SMCX group, although the group did demonstrate a permanent facilitation of recovery as assessed on the beam walking task. The results suggest a behavioral supersensitivity had developed in the contralateral cerebellum when a week or more separation occurred between lesions. Additionally, our preliminary work suggests that in the presence of a right LC and SMCX injury, the left LC appears to be modulating the observed recovery, since subsequent lesioning of the left LC reinstates the hemiparesis on the same side of the body caused by a SMCX injury alone. Supportedby NIH grant R 23 to MGB

462.19 NERVE GROWTH FACTOR (NGF) REGULATES CAPSAICIN-INDUCED CALCIUM UPTAKE INTO CULTURED DORSAL ROOT GANGLION (DRG) NEURONS.
J. Winter* and G. Ryan* (SPON: P. Kirkwood). Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN.

Capsaicin acts selectively on a subpopulation of rat DRG neurons, namely those involved in nociception. *In vivo* capsaicin application causes excitation of these neurons resulting in a painful burning sensation; capsaicin-induced excitation *in vitro* results in accumulation of high concentrations of calcium (measured using $^{45}Ca^{2+}$) into primary cultures of neonatal rat DRG neurons but not sympathetic neurons or non-neuronal cells.

Whereas neonatal DRG neurons require NGF for survival, adult DRG neurons need no growth factors and can be used to study neuromodulatory effects of NGF (eg neuropeptide levels, see Lindsay, Lockett and Winter, this issue).

In response to capsaicin, adult rat DRG neurons grown in the presence of 1μg/ml of NGF took up 4-6 times the amount of Ca^{2+} taken up by neurons grown in the absence of NGF. Approximately 60 p mol of Ca^{2+} were taken up per 1000 NGF-treated neurons during a 10 minute incubation with capsaicin. The amount of Ca^{2+} uptake was dependent on the concentration of NGF and was maximal at 200 ng/ml. Removal of NGF from cultures results in a reduction in responsiveness to capsaicin. The reduced Ca^{2+} uptake in the absence of NGF is not due to loss of capsaicin-sensitive neurons: when NGF is omitted from these cultures for 6 days then added back for 6 days, the level of Ca^{2+} uptake is the same as for 6 day old neurons grown in the continuous presence of NGF. In parallel cultures, the number of neurons that could be killed by capsaicin increased with time in culture, from <10% on the second day, to >30% at day 7.

These results suggest that NGF can regulate DRG neuron responses to capsaicin, possibly by modulation of capsaicin receptors and/or calcium channels on the surface of these cells.

462.20 CORTICAL NOREPINEPHRINE DEPLETION PROTECTS ANIMALS FROM HEMIPARESIS INDUCED BY SENSORIMOTOR CORTEX INJURY.
M.G. Boyeson, K.A. Krobert, and C.M. Grade, Dept. Rehab Med., Univ. Wis. Med. Sch., Madiosn, WI 53706

Previous research (Boyeson & Feeney Neurosci. Abst., 1984) has indicated that a depression in norepinephrine (NE) functioning occurs in the cerebellum contralateral to a sensorimotor cortex (SMCX) injury in rats. The resolution of the NE depression, thought to be regulated by the Locus Ceruleus, may be induced by infusions of NE into the contralateral cerebellum. The NE infusion results in a remarkable recovery from the hemiparesis induced by the SMCX injury. The present experiment suggests that the depression in NE functioning in contralateral cerebellum is a result of damage to terminal LC fibers in SMCX which simultaneously innervate cerebellum. If the hemiparesis following SMCX injury is, in part, mediated by a transient depression in NE functioning in cerebellum induced by damage to the cortical NE projection, then predepletion of cortical NE should serve a protective effect on hemiparesis following SMCX injury. Our results indicate that dorsal bundle lesions followed in 2 weeks by a unilateral SMCX injury results in a very small amount of hemiparesis. Dorsal bundle lesions alone were found to have no effect on hemiparesis as measured by the beam walking task. These results are consistent with the hypothesis that a remote functional depression occurs in the cerebellum contralateral to the SMCX injury that is mediated by the locus ceruleus. In support of this hypothesis is the finding that localized microinfusions of phenoxybenzamine, an alpha receptor antagonist, into the contralateral cerebellum reinstated the hemiparesis in animals long since recovered from the SMCX injury.

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462.21 DIRECT OBSERVATION OF MOTOR NERVE TERMINAL REMODELLING IN LIVING FROGS.
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Signs of synaptic remodelling have been seen in morphological studies of neuromuscular junctions in adult vertebrates (reviewed in Wernig & Herrera, Prog. Neurobiol. 27:251, 1986). At best, however, such a dynamic process can only be inferred from static images. We have overcome this limitation by directly observing nerve terminal remodelling in living animals.

In Oct. 1986 10 Rana pipiens (6-9 g) were anesthetized, chilled, and the deep surface of the surgically exposed sartorius was irrigated for 2 min with 10 μM 4-(4-diethylaminostyryl)-N-methylpyridinium iodide in sterile Ringer. Terminals (4-12 per frog) were visualized by epifluorescence microscopy using high resolution objectives and recorded with a video camera (Dage SIT-66) onto tape. This procedure had no obvious effects on junctional structure. After 110-140 days (mean 27% increase in body wt.), the procedure was repeated and junctions were relocated (92% success rate). After this second observation muscles were excised, stained for the nerve terminal and cholinesterase (ChE), single fibers bearing identified junctions were mounted on slides, and detailed camera lucida drawings were compared with the video images.

Results were analyzed for those terminal branches that were clearly visible at both initial and final times (mean 86% of total terminal length, TTL). Seventy-one of 77 junctions increased in TTL. For all 77 junctions, the net change in TTL was +16±23% (mean±s.d.; range -100 to +125%). For some junctions, this growth was due to simple proportionate enlargement of the terminal, the principal finding from a similar study using the mouse (Lichtman et al., J. Neurosci. 7:1215, 1987). For most junctions, however, disproportionate growth of terminal branches was seen. Moreover, 32 junctions had at least one terminal branch that actually decreased in length, by an average of 10 μm (range 3-51 μm) or 4% (range 1-16%) of TTL. In 5 of these 32 cases, at least one branch completely retracted, sometimes leaving empty ChE-stained post-junctional folds behind. New branches (mean length 10 μm, range 3-79 μm) sprouted from pre-existing branches in 18 junctions (1-8 new branches per junction). In an extreme example, one axon completely retracted from a junction and the site was partially reinnervated by a terminal sprout from the adjacent fiber.

Further analysis of our results should yield information on rates of terminal elongation and retraction, and on the turnover of pre- and postsynaptic specializations. We are continuing these studies using shorter and longer intervals, intermediate in vivo observations, and a different muscle. Possible seasonal differences in remodelling are also being investigated. (Supported by grant NS24805 and RCDA NS00951 from NIH).

463.1 EVIDENCE THAT LHRH AND THE LHRH FRAGMENT, AC-LHRH$^{5-10}$, FACILITATE MATING BEHAVIOR IN THE FEMALE RAT VIA DIFFERENT MECHANISMS. C.A. Dudley and R.L. Moss. Dept. of Physiol. University of Texas Health Science Center, Dallas, TX 75235

Both LHRH and the Ac-LHRH$^{5-10}$ fragment have been demonstrated to facilitate mating behavior in the ovariectomized, estrogen (E)-primed female rat when infused intraventricularly or into specific CNS sites known to be involved in mating behavior. However, the Ac-LHRH$^{5-10}$ fragment does not possess LH-releasing activity *in vitro* or *in vivo*. Electrophysiological studies in which both Ac-LHRH$^{5-10}$ and LHRH were applied to neurons in the ventromedial nucleus of the hypothalamus (VMH) indicate that the two peptides are capable of exerting different effects on firing rate of the same neuron. These findings suggest that the two peptides may achieve their behavioral actions via different mechanisms. This possibility was examined by determining if a potent LHRH antagonist analog produced differential blockade of E+LHRH and E+Ac-LHRH$^{5-10}$ induced sexual behavior in the female rat.

Sprague Dawley female rats weighing 220-240 g at the time of arrival were housed in individual cages in a temperature-controlled room with a reversed light-dark cycle (lights off from 1100h to 2100 h). The animals were bilaterally ovariectomized under ether anesthesia and, two weeks later, were anesthetized with Equithesin and placed in a stereotaxic instrument for bilateral implantation of 23 gauge-stainless steel cannula directed towards the VMH. A preliminary mating behavior test was conducted two weeks later. All animals were primed with 1μg E at 0h and 2.5mg progesterone (P) at 42h. Mating behavior tests were conducted at 48h and consisted of placing the female rat in a semicircular plexiglass mating arena containing two sexually active male rats. The presence or absence of lordotic posture as well as the degree of lordotic arching in response to 15 male copulatory contacts or for 15 min was noted. The results are presented in terms of the lordosis-to-mount ratio (L/M; number of lordoses/number of male contacts). To be included in the study, the animals had to have an L/M of .67 or greater for the E-P test. Two weeks later, the animals were primed with 1μg E at 0 h and infused under ether anesthesia with either the LHRH antagonist, AcΔPro^{1}, pCl, D Phe^{2}, D Trp^{3,6} (100ng/μl; 0.75μl/side) or saline at 46 h. At 46.75 h, the animals were infused with either LHRH or Ac-LHRH$^{5-10}$ (100ng/μl; 0.50μl/side). Mating behavior tests were conducted at 48 h.

Estrogen+LHRH-induced behavior (mean L/M=.71±.08; n=12) was significantly reduced (t=1.92; df=23; p<.05) by prior infusion of the LHRH antagonist (mean L/M=.54±.08; n=13). On the other hand, E+Ac-LHRH$^{5-10}$-induced receptivity (mean L/M=.72±.11; n=13) was not affected by prior infusion of the LHRH antagonist (mean L/M=.71±.08; n=12). Thus, competitively binding sites at which LHRH acts did not interfere with the ability of Ac-LHRH$^{5-10}$ to enhance mating behavior. These results support the hypothesis that the LHRH fragment and the LHRH decapeptide influence mating behavior via different mechanisms. Supported by NIMH grant # MH41784.

463.2 FUNCTIONAL CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS IN THE NEONATAL RAT SPINAL CORD: EVIDENCE FROM AUTORADIOGRAPHIC AND ELECTROPHYSIOLOGICAL STUDIES. J.A. Bell* and E.B. De Souza (SPON: G. Battaglia). Neuroscience Branch, NIDA Addiction Research Center, Baltimore, MD 21224.

Accumulating evidence suggests that CRF may play a role as a neurotransmitter in the CNS to coordinate the organism's endocrine, behavioral and autonomic responses to stress. The autonomic responses to CRF appear to be mediated primarily through actions of this neuropeptide in brain; however, some preliminary data suggests that CRF may act within the spinal cord to produce some of its parasympathetic effects. The isolated spinal cord of the neonatal rat provides a good model for studying spinal mechanisms involved in the action of neuropeptides. In the present study, we have localized CRF receptors using autoradiographic techniques and studied the actions of CRF in the neonatal spinal cord preparation using electrophysiological methods. CRF receptors were present in highest concentrations in the superficial layers (laminae I and II) of the dorsal horn with progressively lower concentrations in motoneurons of the ventral horn and in the intermediate and central zones of the neonatal spinal cord, respectively. In electrophysiological studies, extracellular dorsal and ventral root recordings were obtained from an isolated hemisected neonatal spinal cord preparation. Rat/human (r) and ovine (o) CRF depolarized the ventral root in a concentration related manner. The depolarizing response to CRF was mediated by pharmacologically specific receptors since the putative receptor antagonist, alpha helical oCRF(9-41) partially blocked the depolarizing response to CRF; the inactive analogues oCRF(1-22)OH and oCRF(7-41) had no effect, whereas the active analogue Nle^{21,38} rCRF caused a depolarizing response comparable to CRF. The depolarizing response to CRF comprised two components, a rapid depolarizing response that reached maximum amplitude during the CRF superfusion and a slow depolarizing response which continued for 10 min after cessation of CRF. In reduced calcium concentration sufficient to block synaptic transmission, the rapid segment of the depolarizing response to CRF was abolished while the slow depolarizing response was maintained. The data suggest that the initial, rapid depolarizing response to CRF is mediated by activation of excitatory interneurons, probably through actions of CRF on receptors in the superficial layers of the dorsal horn, while the subsequent slow depolarizing response represents a direct action of CRF on receptors on motoneurons in the ventral horn. In summary, the electrophysiological responses to CRF correlate well with the distribution of receptors within the spinal cord and suggest that CRF may play a role in regulating spinal cord reflex function.

463.3 PRESSOR RESPONSES TO AMASTATIN, BESTATIN AND AII ARE SUPPRESSED BY PRETREATMENT WITH ANGIOTENSIN ANTAGONIST SARTHRAN. C.M. Batt, E.W. Klein*, J.W. Wright, J.W. Harding. Departments of Psychology and VCAPP, Washington State University, Pullman WA 99164-4830

The octapeptide angiotensin II (AII) has generally been considered the active peptide of the central renin angiotensin system. However recent reports have found intracerebroventricularly (icv) applied angiotensin III (AIII, heptapeptide) to be equipotent with AII as a pressor agent (Fink et al., 1985; Wright et al., 1985). Utilization of microwave fixation to stop peptidase activity in conjunction with HPLC analysis has also recently shown that centrally injected iodinated AII has a half-life of 23 sec as compared with 8 sec for AIII (Harding et al., 1986). An analysis of structure-activity relationship and signal termination at the angiotensin receptor is important to an understanding of angiotensin-induced increases in blood pressure. Amastatin (AM) and bestatin (BE) are aminopeptidase inhibitors which may help determine the mechanisms of signal termination. The tetrapeptide AM inhibits the action of aminopeptidase A which cleaves AII to AIII; the dipeptide BE inhibits the action of aminopeptidase B which cleaves AIII to the biologically inactive hexapeptide (Umezawa et al., 1976, 1978; Rich et al., 1984). Additionally both aminopeptidase inhibitors act on the leucine aminopeptidases, and BE may also have an effect on aminopeptidase A (Umezawa et al., 1976). Central icv administration of AM or BE has been shown to increase blood pressure in rats (Jensen, 1987).

In the present investigation we addressed the following questions: 1) Are these inhibitory effects dependent on the central angiotensinergic system? 2) How long do bolus icv injections of AM and BE inhibit the angiotensinergic system? The first question was answered by utilizing the specific angiotensin receptor antagonist sarthran [Sar^{1},Thr^{8}-AII], and we found that AM-, BE-, and AII-induced pressor responses were prevented. After an initial bolus injection of artificial cerebrospinal fluid (aCSF, 2 ul) or sarthran (10 nmoles in 2 ul aCSF), additional injections of AM (10 or 100 nmoles in 2 ul aCSF) or BE (10 or 100 nmoles in 2 ul aCSF) or AII (10 or 100 pmoles in 2 ul aCSF) were given at 30, 60 and 90 minutes. The results indicate that sarthran is effective in suppressing normal pressor responses to AM, BE and AII. Recovery from the inhibitory effects of sarthran was evident at the 90-minute timepoint. Since sarthran is a specific angiotensin antagonist, and since it is effective in suppressing pressor responses to AII, AM and BE must therefore be acting on the angiotensin system rather than some other peptide system.

The results indicate that AM and BE are having their pressor effects via the central angiotensinergic system and that the patterns of AM, BE and AII recovery from the influence of a specific angiotensin receptor antagonist are very similar.

463.4 INCREASED BLOOD PRESSURE INDUCED BY CENTRAL APPLICATION OF AMINOPEPTIDASE INHIBITORS IS ANGIOTENSINERGIC DEPENDENT. L.L. Jensen, J.W. Harding, and J.W. Wright. Departments of Psychology and VCAPP, Washington State University, Pullman, WA 99164-4830

Intracerebroventricular (icv) injections of angiotensin II (AII, octapeptide) and angiotensin III (AIII, heptapeptide) are equipotent as pressor agents (Fink et al., 1985; Wright et al., 1985), despite the fact that AIII is metabolized in the brain of the normotensive rat at a much faster rate than AII (Harding et al., 1986). The relative importance of central AIII is supported by results from microiontophoretic application of AII and AIII in the subfornical organ and paraventricular nucleus of the hypothalamus of rats that indicate much greater neural activity induced by AIII than AII (Felix and Schlegel, 1978; Harding and Felix, in press). Recently our laboratory determined that pretreatment with bestatin (BE) significantly extended the half-life of icv administered iodonated AIII and to a lesser degree AII in Sprague-Dawley rats, and significantly potentiated icv AII and AIII induced pressor effects in SHR as compared with WKY and Sprague-Dawley rats (Wright et al., 1985).

The present investigation evaluated the efficacy of these inhibitors with respect to icv induced pressor responses in these three rat strains. BE was found to elevate blood pressure in a dose response fashion in members of all strains with the SHR indicating greatest responsiveness. Treatment with amastatin (AM) also induced pressor elevations in a dose response pattern but was less potent than BE. Pretreatment with a specific angiotensin receptor antagonist, Sarthran (Sar^{1},Thr^{8}-AII), significantly reduced subsequent angiotensin induced pressor responses suggesting that AM and BE are having their pressor effects via the central angiotensinergic system rather than other peptidergic pathways.

These results indicate that aminopeptidase inhibitors can be successfully employed as probes to manipulate peptidergic systems and support the hypothesis that AM and BE are having their pressor effects via the endogenous central angiotensinergic system. The results further suggest that the increased half-life of AIII via icv BE treatment resulted in significantly elevated pressor responding as compared with blood pressure elevations due to the increased stability of AII accompanying icv AM infusion. Thus, these findings support an important role for AIII in the central control of blood pressure in hypertensive and normotensive rats. However, because these inhibitors are not as specific when applied in vivo as they appear to be in vitro, a definitive explanation of the present findings must await further investigation. Supported by NIH Grants HL32063 and TW01112, the American Heart Association, and the American Heart Association of Washington State.

463.5 SINGLE-PULSE STIMULATION OF THE MEDIAL PERFORANT PATHWAY WITH CONCURRENT ADMINISTRATION OF CCK 8-S APPEARS TO POTENTIATE THE RAT DENTATE GYRUS EAP. Dennis Dahl. Research Department, CIBA-GEIGY Corp., Summit, NJ 07901 and The Rockefeller University, NY, NY 10021.

Long term Potentiation (LTP) is defined as a use-induced increase of synaptic efficacy. Because of its exemplary induction in the pathway from the medial entorhinal cortex (perforant pathway [PP] to the dentate gyrus (DG), LTP is most often studied in the excitation mode. Significant and persistent increment in the amplitudes of the excitatory post-synaptic potential (EPSP) and, less often studied, the evoked action potential (EAP) are taken as indicators of LTP induction.

It has been previously reported (Dahl and Sinton, Fed. Proc., 46, 3, 1987) that cholecystokinin, as the sulphated octapeptide CCK 8-S),has a dose-dependent excitatory effect on DG granule cells that is manifested in an increase in the amplitude of the EAP. In the experiments reported here, performed on male Sprague-Dawley rats (Harlan Farms), single doses of CCK 8-S (5.0 μg/Kg, i.v.t.) were applied with concurrent single-pulse stimulation to the PP (interpulse interval 15"). Recordings taken in the molecular layer of the DG revealed that the EPSP amplitude first decreased with CCK 8-S administration ($\bar{X}$=25%), then increased to and was stable at the control level, and decreased again ($\bar{X}$=30%) within a one hour period during which the EAP increased in amplitude ($\bar{X}$=50%) with the initial decrease of the EPSP and then remained stable at that level.

Administration of CCK 8-S may provide the conditions of depolarization of granule cells that coupled with stimulation of the PP is necessary for induction of potentiation. Once at its elevated level, the EAP appears to be independent of EPSP amplitude. An increase in EPSP is not seen under these conditions, perhaps, because volleys of stimulating pulses to the PP are not required. If these results are exemplary of LTP, then increased "synaptic efficacy" often spoken of as the basis of LTP may not necessarily be manifested in a larger EPSP amplitude.

463.6 SECRETIN FAMILY PEPTIDES ENHANCE SYNAPTIC TRANSMISSION IN RAT SYMPATHETIC GANGLIA. D.G.MCKENNA*, D.A.MCAFEE, C.A.BRIGGS; Beckman Res. Inst. Duarte, CA, Abbott Labs, Chicago, IL, Nelson Research, Irvine, CA, 92715.

Neuropeptides appear to play a role in a number of autonomic functions. These substances raise cyclic 3',5' adenosine monophosphate (cAMP) levels in various tissues including autonomic ganglia (Ip, N.Y. et al, J. Neuroscience 5:1947, 1985). Forskolin, an adenylate cyclase activator, and isoproterenol, a beta adrenergic agonist, both activate the formation of cAMP in nervous tissue. They also enhance nicotinic transmission and the release of endogenous acetycholine (ACh) from sympathetic ganglia. We, therefore, set out to determine if peptides of the SECRETIN family similarly enhance ganglionic transmission.

Superior cervical ganglia (SCG), isolated from adult Sprague Dawley rats, were placed in temperature controlled 25°C nerve chambers and bathed in oxygenated Lockes medium. Sufficient nicotinic (d-tubocurarine) and muscarinic (atropine) antagonists were added to the perfusate to reduce maximal nicotinic synaptic response by 50%, and to totally block muscarinic synaptic responses. Single, supramaximal stimuli were delivered to the cervical sympathetic nerve at a frequency of 1/minute. The resulting post-synaptic compound action potential, recorded from the internal carotid nerve, was digitized, and the amplitude and integral were measured. Synaptic efficacy was assumed to be related to the magnitude of this postsynaptic potential.

Brief (5 min) exposure to low concentrations (10-100 nM) of SECRETIN, VIP (vasoactive intestinal polyptide), and PHI (peptide histidine-leucine) enhanced synaptic efficacy for long periods (30-60 min). The effect of these peptides was dose dependent and was abolished by pretreatment with trypsin. An unrelated peptide, BRADYKININ, had no effect on synaptic efficacy. The order of potency was SECRETIN > VIP > PHI.

Similarly, brief exposure to forskolin and isoproterenol (3 uM) produced a similarly long-term enhancement of synaptic efficacy. These results suggest that one mechanism underlying long-term changes in synaptic efficacy may be dependent on cAMP. (Supported in part by PHS Grant # NS18966.)

463.7 INSULIN'S CENTRAL GLUCOREGULATORY ACTION IS MEDIATED BY A PHOSPHOLIPID-DEPENDENT MECHANISM. S. Amir and Y. Shechter*. Neurosci. Ctr. and Dept. of Hormone Res., Weizmann Inst. Sci., Rehovot, Israel.

Insulin interacts with glucoregulatory mechanisms in the central nervous system (CNS); when injected centrally in experimental animals it rapidly lowers the levels of circulating glucose by inhibiting glucose output from the liver through action upon the parasympathetic outflow. This central effect is initiated by binding of hormone to specific receptors exhibiting binding characteristics resembling those described for insulin in peripheral tissues. However, the post binding events mediating the central glucoregulatory action of insulin are unknown. Polymyxin B (PMB) is a cyclic polycationic decapeptide that can block insulin-stimulated glucose uptake and its subsequent metabolism in muscle and adipose tissues by an effect at a level proximal to the hormone binding sites, without affecting interaction of the hormone with membrane receptors (Amir et al., J. Biol. Chem., in press). In the present investigation, intracerebroventricular (ICV) injection of 0.5 μg insulin produced rapid, transient hypoglycemia in male ICR mice (18-31 g), which was completely blocked by the coadministration of 5 μg PMB (Sigma). A similar dose of PMB given intravenously had no effect on the hypoglycemic response to central insulin. Also, central PMB (5 μg) had no effect on plasma glucose when administered alone. The central anti-insulin action of PMB was dose-dependent (1-25 μg), and specific; colistin A (polymyxin E, Rafa 1-25 μg), which differs from PMB by one conservative amino acid substitution in the ring structure failed to block the central hypoglycemic effect of insulin. Finally, the anti-insulin action of PMB appeared to depend on interaction of the peptide with membrane phospholipids, since acetylation of all five amino groups involved in peptide-phospholipid interaction, or preincubation of PMB with excess exogenous phospholipids, phosphatidylcholine, phosphatidylethanolanine or lipid A, diminished the inhibitory effect. Collectively, the results show that PMB blocks in a potent and specific manner the central effect of insulin on plasma glucose by interacting with neuronal phospholipids. Previous studies have shown that insulin activates membrane bound Ca^{2+} - and phospholipid-dependent protein kinase (PKC) and that PMB is a selective antagonist of PKC in various tissues, including brain. We therefore consider the possibity that activation of Ca^{2+} - and phospholipid-dependent protein kinase by insulin may be a critical post binding event involved in the central effect of the hormone on circulating glucose.

463.8 EFFECT OF ANGIOTENSIN II AND PEPTIDE YY ON CEREBRAL AND CIRCUMVENTRICULAR BLOOD FLOW. U.I. Tuor*, M.H. Kondysar* and R.K. Harding* (SPON: A. Krantis). Department of Physiology, University of Ottawa, 451 Smyth Rd., Ottawa, Canada K1H 8M5.

Angiotensin II and peptide YY (PYY) are putative neuro/humoral agents acting at several circumventricular regions in the brain. These peptides also constrict cerebral vessels in vitro or in situ and therefore may influence blood flow in the circumventricular organs - regions which lack a blood brain barrier. We examined the effect of intravenous infusion of saline, angiotensin II and PYY on local cerebral blood flow in 8 circumventricular and 20 non-circumventricular brain regions. Local cerebral blood flow was measured 15 minutes following the onset of peptide infusion by quantitative 14C-iodoantipyrine autoradiography in 17 conscious rats.

Angiotensin II and PYY infusion elevated total peripheral resistance, the mean arterial blood pressure being increased by 24±3% and 14±3%, respectively. However, blood flow in all 28 brain regions examined was not reduced in comparison with saline infused controls. Thus, angiotensin II and PYY did not produce a vasoconstriction sufficient to decrease blood flow in either the non-circumventricular or circumventricular brain regions.

Differences between angiotensin II and PYY infused groups were observed. During PYY infusion, blood flow was increased and cerebrovascular resistance was decreased in the circumventricular regions whereas blood flow in the 20 non-circumventricular regions remained rather constant. The elevations in circumventricular blood flow were most pronounced in the pineal body (60±11% control) and in the area postrema (44±11% control). During angiotensin II infusion, local cerebral blood flow in all regions examined was unaltered whereas local cerebrovascular resistance was elevated in response to the increase in arterial blood pressure (ie. a local autoregulation of blood flow). The increases in local cerebrovascular resistance were most pronounced in the subcommisural organ (33±14% control), choroid plexus (25±8% control) and subfornical organ (26±7% control). Thus, the response of the cerebral circulation to angiotensin II and PYY infusion differed markedly. This is likely due to differential effects of these peptides on local cerebral metabolism (eg. increases in metabolism during PYY infusion) and on local vascular resistance (eg. a relative vasoconstriction during angiotensin II infusion).

The local cerebral circulatory response to peptide administration was dependent on the location of the region examined (circumventricular or non-circumventricular) and on the vasoactive peptide infused.

463.9 POTENTIATION OF BRADYKININ-INDUCED NOCICEPTION BY ANGIOTENSIN CONVERTING ENZYME INHIBITORS. R.G. Browne. Research Department, Pharmaceuticals Division, CIBA-GEIGY Corp., Summit, NJ 07901

Bradykinin is an endogenous algesic mediator which elicits pain upon intradermal injection in man. In guinea pigs, the close-arterial injection of sub-microgram quantities of bradykinin elicits a brief vocalization response which can be blocked by the prior administration of various analgesic drugs (Adachi and Ishii, J. Pharmacol. Exp. Ther. 209: 117-124, 1979). Bradykinin is rapidly metabolized by at least two kinases, and one of these enzymes (kinase II) has been shown to be inhibited by the angiotensin converting enzyme (ACE) inhibitor captopril (Regoli and Barabe, Pharmac. Rev. 32: 1-46, 1980). In fact, some side effects seen in man following administration of captopril have been attributed to increased bradykinin actions (Sesko and Kaneko, Arch. Intern. Med. 145: 1524, 1986). The present experiment was designed to test the hypothesis that kinase II inhibition by captopril would result in a potentiation of the nociceptive effects produced by intra-arterial bradykinin injection.

Male Duncan Hartley guinea pigs (ELM:(DH)) weighing 300-350 g were maintained in single cages under standard laboratory conditions. Medical grade polyvinyl catheters (0.20 mm i.d. x 0.50 mm o.d., Dural Plastics) were implanted, under ketamine (Vetalar, Parke-Davis) anesthesia, into the right femoral artery. At least 48 hrs after surgery the guinea pigs were administered varying concentrations of bradykinin (Bachem) in a volume of 0.1 ml followed by flushing the catheter with 0.3 ml of saline. The animals were individually observed for the presence of a brief (less than 60 sec duration) vocalization response characterized by at least three clearly audible "squeals" over the two min period following the injection. For experiments involving drug interactions, 200 ug of either captopril (Squibb) or enalapril (Merck) were injected into the arterial catheter in a volume of 0.1 ml 30 min before re-testing for the presence of vocalization with bradykinin.

The results of this study demonstrate a marked potentiation of bradykinin's nociceptive actions by the ACE inhibitors captopril and enalapril. In control guinea pigs, vocalization was observed at sub-microgram doses (threshold doses for individual animals ranged from 0.025 to 0.25 ug). Captopril pre-treatment shifted the dose response for bradykinin-elicited vocalization to the left. In fact, following captopril, greater than half the animals were observed to emit a vocalization response at previously sub-threshold concentrations of bradykinin. These results support the role of kinase II in the degradation of bradykinin and are the first to demonstrate a potentiation of bradykinin-induced nociception by ACE inhibitors.

463.10 LONG TERM CHANGES IN SYNAPTIC ACTIVITY INDUCED BY THE MAST CELL DEGRANULATING PEPTIDE IN CA1 AND CA3 HIPPOCAMPAL NEURONES

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The mast cell degranulating peptide (MCD) which has been isolated from bee venom, induces theta rhytm in the hippocampus and paroxysmal activity upon central administration in rats (Bidard et al., Brain Res. 1987, in press). These effects are mediated by high affinity binding sites concentrated in cortical structures, notably in the hippocampus (Ibid). The present experiments were performed to study the mechanism of action of MCD.

In rat hippocampal slices, a brief application (2-3 min) of MCD (0.5-2 μM) induced in CA1 a long lasting enhancement (> 3 hours) of the field and intracellular epsp produced by Schaffer collateral stimulation. The afferent volley and the post synaptic cell excitability were not changed by MCD. The action of MCD is therefore reminiscent of the long term potentiation (LTP) produced by a train of high frequency electrical stimulation (Cherubini et al., Nature, 1987, in press).

In the CA3 region, MCD (0.5 - 2μm) produced spontaneous bursts that persisted for several hours. These bursts are network driven (not endogenous pacemaker bursts) and the underlying paroxysmal depolarizing shift is a giant epsp with a reversal potential close to 0 mV. Furthermore, following application of MCD, stimulation of the mossy fiber or commissural pathways evoked a conventional epsp followed by an evoked network burst (ENB). The ENB which could be induced for several hours, was not blocked by bicuculline or elevated divalent cation concentrations. Concomittant, but not subsequent applications of TTX (1 μM) or cobalt (2 mM) blocked the long lasting effects, suggesting that the action of MCD requires synaptic activity.

Therefore, MCD produces long lasting changes in the synaptic response both in CA1 and CA3 region. It is possible that acting presynaptically, MCD releases protein(s) and/or amino acids which will act postsynaptically to produce the long lasting changes. Since an endogenous ligand to MCD has been found in brain extracts with receptor and radio immuno assays (Cherubini et al., Nature, 1987, in press) the present results suggest that a MCD like agent may be involved in the long lasting changes in synaptic acitivity.

M. Gho is a fellow of Fondation Fyssen.

463.11 cGMP, A POSSIBLE SECOND MESSENGER MEDIATING THE EFFECT OF FMRFAMIDE ON APLYSIA NEURON R14. M. Ichinose* and D.J. McAdoo. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

The molluscan cardioexcitatory peptide FMRFamide (Phe-Met-Arg-Phe-amide) induces inward current in voltage clamped neuron R14 in the abodminal ganglion of Aplysia californica (Ichinose and McAdoo, Neurosci. Abstr. 12: 149, 1986). Pressure ejected FMRFamide (200 μM, several nl) produced a slow inward current which maximized after 30-60 sec and lasted about 3 min. The long latency and long duration of the effect of FMRFamide on R14 suggest that a second messenger mechanism is involved. Cyclic nucleotide mediation for FMRFamide response was suggested because the FMRFamide response was enhanced by perfusion with phosphodiesterase inhibitors, IBMX (1 mM) or theophylline (1 mM). Possible second messengers of FMRFamide were investigated. Cyclic AMP (cAMP) and cyclic GMP (cGMP) were injected into the neuron and the response recorded by means of a two-electrode voltage clamp system. A cAMP-induced current that was similar to the response of R14 to FMRFamide has been reported in other molluscan neurons (Conner and Hochberger, J. Physiol. 354: 139-162, 1984). However, cAMP (100 mM, several pl) injected into R14 induced a dose-dependent and sustained outward current (up to 10 nA, almost 30 min). When cGMP (50 mM, several pl) was injected into the neuron, an inward current similar to the FMRFamide response was observed. The cGMP induced inward current increased with depolarizing holding potentials from -70 mV, maximizing at about -30 mV. Partial replacement of sodium with N-methyl-D-glucamine decreased the cGMP-induced inward current, sodium free solution eliminated the inward current. Changing the external potassium concentration did not change the cGMP response. Replacing external chloride with gluconate increased the cGMP induced inward current (in one-fifth chloride solution to 4.3 times the response in normal artificial seawater, n=3), and the reduction of external calcium enhanced the cGMP-induced response (in one-fifth calcium solution to 2.7 times the response in normal artificial seawater, n=3). The Na-dependency, K-independency and inhibitory actions of Ca and Cl on the cGMP induced inward current and on the FMRFamide induced inward current were similar. Furthermore, when a few drops of the guanylate cyclase activator, nitroprusside (50 mM) was applied to the bath, an inward current similar to the FMRFamide response was observed. These results suggest that cGMP mediates the FMRFamide response in R14.

463.12 GROWTH HORMONE RELEASING HORMONE COMPLEMENTARY PEPTIDES. B. Kacsoh†*, W.R. Crowley††, G.V. Shah†*, T.G. Cooper†††*, R.A. Sumrada†††*, and C.E. Grosvenor†. Depts. of Physiology†, Pharmacology††, and Microbiology†††, Univ. of Tenn., Memphis, TN 38163.

We synthesized two peptides encoded by a hypothetic mRNA sequence, complementary to the mRNA for rat growth hormone releasing hormone (GHRH). One peptide was encoded by the complementary mRNA in the 3'-5' direction (3'-5' complementary peptide; 3'-5' CP) and is complementary to the amino acid residues 14-25 of GHRH. The other peptide was translated in the 5'-3' direction (5'-3'CP) and is complementary to GHRH (18-43). Hence, both peptides overlap the sequence complementary to GHRH (18-25). The 5'-3'CP was conjugated with keyhole limpet hemocyanin, and rabbits were immunized with the conjugated peptide. The antiserum generated has a high titer and no significant cross reactivity with GHRH, 3'-5'CP, glucagon, LHRH and growth hormone (GH). The IgG fraction of the antiserum was purified by affinity chromatography. The biological activity of GHRH, 3'-5'CP, 5'-3'CP and the IgG raised against the 5'-3'CP (5'-3'CPIgG) were tested in primary pituitary cell culture on GH secretion. GHRH, 3'-5'CP and 5'-3'CPIgG stimulated GH secretion, while the 5'-3'CP itself did not elevate basal GH secretion [basal GH secretion: 624 ± 56 ng/ml; GHRH (10^{-8}M): 1247 ± 90; 3'-5'CP (10^{-8}M): 1458 ± 200; 5'-3'CPIgG (0.1 μg/ml): 1296 ± 247; 5'-3'CPIgG (100 μg/ml): 1944 ± 193; 5'-3'CP (10^{-5}M): 522 ± 50 ng/ml]. The overlapping parts of the two CPs match the hydropathic pattern of GHRH (22-27). Along with the data of Ling et al. (Biochem. Biophys. Res. Comm. 123:854-861, 1984) our results suggest that GHRH (22-27) is a domain of GHRH involved in the receptor ligand interaction. [Supported by grants to CEG: USPHS HD-04358 and the University of Tennessee Molecular Resource Center]

463.13 ERGOT ALKALOIDS BLOCK NEUROGENIC PLASMA EXTRAVASATION IN THE DURA MATER: PROPOSED MECHANISM IN THE TREATMENT OF VASCULAR HEADACHES. K. Saito, S. Markowitz, M. Moskowitz. Neurosurgery & Neurology, Massachusetts General Hospital, Boston MA 02114.

First introduced for the treatment of migraine and cluster headaches more than 50 yrs ago, the ergot alkaloids remain the most effective drug therapy for headaches. Although the ergots are presumed to work by constricting painful, dilated cephalic vessels, evidence is lacking to support fully either a vasodilator mechanism of headache pathogenesis or a vasoconstrictor mechanism of drug action. In this report we provide evidence that plasma extravasation (PE) which develops within the dura following depolarization of the trigeminal (V) nerve is blocked by ergot alkaloids by a mechanism that is C-fiber dependent.

Our first studies demonstrated that PE developed within the dura following capsaicin (i.v.) or electrical V stimulation in normal adults. Sprague Dawley rats (n=100) were first injected with ^{125}I-BSA and 5 minutes later either administered capsaicin i.v. (0.1 umoles) or unilaterally stimulated electrically using bipolar electrodes (1.2 mA, 5 msec, 5 Hz) following bilateral electrode insertion. Within 10 minutes, the animals were perfused and the dura mater removed for analysis of isotope content. The development of enhanced permeability was dependent upon the presence of unmyelinated C-fibers in as much as PE was not measurable in adult animals in whom C-fibers were depleted by capsaicin treatment as neonates. Moreover, acute pretreatment of animals with dihydroergotamine (DHE), ergotamine tartrate (ET) or chronic methysergide blocked the capsaicin response in doses similar to those used clinically. All three drugs were also effective against electrical stimulation, although at higher doses.

To support a role for the neuropeptides as mediators of neurogenic PE in the dura mater, and to evaluate the role of vasoconstriction as a possible mechanism of ergot action, the ability of sensory peptides to induce PE was investigated. SP, NKA but not CGRP enhanced permeability in the dura mater, an effect that was C-fiber independent and largely the result of a direct vascular action. Neither ET or DHE pretreatment blocked the SP or NKA-induced response even when administered in amounts much greater than that required to block capsaicin-induced PE. This observation coupled with the demonstration that two potent vasoconstrictors angiotensin II or phenylephrine did not block capsaicin-induced PE suggests that vasoconstriction is not the major mechanism by which ergot alkaloids block neurogenic PE and rather raise the possibility that ergot compounds act directly to alter the ability of the sensory nerves to promote PE, perhaps by interfering with the release of mediators in much the same way as has been suggested in the case of opiate peptides.

463.14 LATERAL HYPOTHALAMIC DAMAGE AND NEUROPEPTIDES: EFFECTS ON GASTRIC FUNCTIONS AND BLOOD GLUCOSE LEVELS. C.V. Grijalva, M.W. Gunion, and B. Roland. Dept. of Psych. and Med., UCLA, Los Angeles, CA 90024; and V.A. Med. CTR, Sepulveda, CA 91343.

Intracisternal (i.c.) injections of bombesin in rats causes hyperglycemia, suppression of gastric acid secretion (Taché, et al., Proc. Natl. Acad. Sci. USA 77:5515, 1980), and the attenuation of stomach erosions induced by cold-restraint stress (Taché, et al., Life Sci. 24:1719, 1979. Corticotrophin-releasing factor (CRF) also induces hyperglycemia and suppresses gastric secretion when administered into the brain (Taché et al., Science 222:935, 1983). The present study was conducted to examine; (1) whether either bombesin or CRF would prevent the formation of stomach erosions induced by lateral hypothalamic (LH) lesions in rats and, (2) the interactive effects of CRF administration and LH lesions on gastric acid secretion, and serum gastrin and glucose levels.

In one experiment 5 groups of 9 male rats were matched for body weight. All groups were food deprived overnight, anesthetized with sodium pentobarbital, and then 4 groups were given bilateral electrolytic LH lesions. The fifth group was given sham surgery. Immediately following surgery the LH groups were injected (i.c.) with either bombesin (500 ng), rat or ovine CRF (10 µg), or saline vehicle (10 µl). All groups were food and water deprived for 24 h post-operatively, sacrificed by decapitation, and the stomachs examined. Mean total area (mm^2) of gastric damage was as follows: Sham-Veh (1.15), LH-Veh (23.2), LH-ovCRF (15.7), LH-rtCRF (14.8), LH-Bomb (6.1).

In the second experiment, groups of rats were either sham operated of given LH lesions under methohexital anesthesia, injected with either rat-CRF (10µg) or vehicle, and then the pylorus ligated. Two hours later all rats were decapitated and gastric secretion and trunk blood collected. Group mean values are presented in the table below:

Group	N	Vol/2h	pH	Acid Output (µEq/2h)	Gastrin (pg/ml)	Glucose (mg/dl)
Sham-Veh	8	2.63	1.4	279.7	70	104.6
Sham-CRF	9	0.88	4.1	27.2	100	190.3
LH-VEH	10	3.04	1.3	326.9	76	123.3
LH-CRF	9	0.93	1.7*	63.7	88	126.9

*(N=7)

The results show that bombesin attenuated the formation of gastric erosions after LH lesions ($p<.05$), whereas CRF did not. However, CRF effectively suppressed gastric acid output in LH rats ($p<.01$). These results indicate that bombesin provides protection against gastric erosions by different mechanisms. The results also show that LH lesions prevented the hyperglycemic effects of CRF, reinforcing the idea that the LH area is a primary site of action for glucose homeostasis.

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463.15 REGULATORY PEPTIDE CONCENTRATIONS IN HUMAN SYNOVIAL FLUID OBTAINED FROM ARTHRITIC KNEE JOINTS. D. Roddy*[1], W. Ginsburg*[2], J. Bailey*[1], D. Lucas*[1], A. Morris*[1], W. Reilly*[1], R. Valente*[2], V.L.W. Go*[1] and T.L. Yaksh*[3] (SPON: P.J. Dyck). [1]Gastroenterology Unit, [2]Dept. of Rheumatology and [3]Dept. of Neurosurgical Res., Mayo Clinic, Rochester, MN 55905.

Recent studies have suggested that neuropeptides in peripheral tissue may modulate afferent neural activity and proliferation of lymphocytes and synoviocytes. To determine whether differences in synovial concentrations of regulatory peptides exist in arthritic diseases, we determined concentrations of peptides in synovial fluids obtained from patients with inflammatory (rheumatoid arthritis: RA; polymyalgia rheumatica: PMR; psoriasis: P; and idiopathic: I); and non-inflammatory (degenerative joint disease: DJD) diseases. Synovial fluids were obtained from patients undergoing arthrocentesis of the knee, transported on ice, centrifuged and stored at -20°C. These immunoreactive regulatory peptides were determined by specific radioimmunoassay: bombesin (BLI), cholecystokinin (CCK), neuropeptide Y (NPY), substance P (SP), human pancreatic polypeptide (HPP), motilin (MOT), gastrin (G) and pancreatic glucagon (PG).

Results:

	WBC/mm^3	Immunoreactive Peptides (pg/ml; mean ± SEM) BLI	CCK	NPY	SP	HPP	PG	MOT	G
RA (n=3)	12,057 ±4,068	132 ±38	58 ±20	630 ±190	72 ±20	128 ±56	137 ±4	59 ±9	495 ±138
PMR, P, I (n=7)	18,261 ±6,112	43 ±6	83 ±41	711 ±214	46 ±10	101 ±25	194 ±40	109 ±20	277 ±56
DJD (n=5)	377 ±189	31 ±5	41 ±15	490 ±80	28 ±8	295 ±80	106 ±23	59 ±9	212 ±33
pANOVA*	<.01	<.01	NS	NS	NS	<.05	NS	NS	<.05

*One-way analysis of variance among the 3 groups.

In RA, concentrations of BLI, SP and G appeared increased compared to DJD and other non-RA inflammatory disorders, and HPP was decreased compared to DJD. In non-RA inflammatory disorders, CCK, MOT, and PG were increased and HPP was decreased compared to DJD.

Conclusions: The relevance of increased BLI, SP and G to the pathophysiology of RA remains to be determined. The regulatory peptides in synovial fluid may be released locally by synoviocytes and/or WBC, or they may be the result of hemofiltration due to increased permeability of the synovial membrane. This remains to be elucidated. (Supported by NIH grant AM-34988, TLY.)

463.16 NEUROPEPTIDE LEVELS IN MALE AND FEMALE HUMAN PLASMA AS A FUNCTION OF AGE. A. Morris*, S. Michener*, D. Roddy*, J. Bailey*, D. Harteneck*, D. Lucas*, W. Reilly*, V.L.W. Go* and T.L. Yaksh*[1] (SPON: J.D. Grabow) GI Hormone Research Laboratory and [1]Neurosurgery Research Lab., Mayo Clinic, Rochester, MN 55905.

Current evidence emphasizes the presence of a variety of neuropeptides in peripheral tissues. Organic dysfunction may be mirrored in changes in the circulating levels of these agents. The present studies seek to concurrently establish baseline profiles of these hormones in the plasma of normal males and females, and to determine the relationship of these levels to age. Sufficient EDTA-plasma was obtained after overnight fasting in a volunteer blood donor population of clinically normal subjects. Samples were aliquoted and assayed in duplicate using radioimmunoassays for 18 peptides. Parallel dilution curves with plasma samples were obtained for all assays where measurable levels were observed. Regression analysis of the results indicated that: 1) significant ($P<0.05$) increases with age were noted for HPP in males and for PHM, HPP, G-CCK, CCK$_{33}$ and CCK-8 in females; 2) significant decreases with increasing age were noted for PYY and NPY in males and NPY in females; 3) no reliable difference between male and female hormone levels were noted between any age group ($P>0.10$). (Supported by NIH grant AM-34988, TLY.)

PEPTIDE	ASSAY ANTIBODY NUMBER	ABSOLUTE SENSITIVITY (pg/ml)	MALE (X ± SE; pg/ml) Young No.: 20 Age: 22-30	MALE Old 8 44-59	FEMALE Young 9 23-31	FEMALE Old 10 43-59
Bombesin (BLI)	K388	10	52±7	43±5	69±10	51±7
C-Peptide (CP)	Im x 600	160	1958±320	2509±466	1520±166	1674±169
Cholecystokinin-8 (CCK8)	216	8	9±1	8±0.8	9±1	30±12
Cholecystokinin-33 (CCK33)	3482	100	74±6	73±8	59±6	86±8
Gastrin CCK (GCCK)	3438	20	64±6	71±6	62±9	84±5
Gastrin Specific (G-SP)	4817	20	218±10	220±10	227±13	248±16
Gastrin Inhibitory Peptide (GIP)	4888	60	208±31	253±24	144±15	233±43
Glucagon-like Immunoreactants (GLI)	K4023	20	544±117	374±55	328±40	312±38
Met-Enkephalin (Met)	K382	50	<50±--	<50±--	<50±--	<50±--
Motilin (Mot)	GP71	50	124±14	169±36	143±24	178±29
Neuropeptide Y (NPY)	221	200	598±28	487±32	675±66	483±40
Neurotensin (NT)	K398	15	100±10	72±8	84±8	82±7
Pancreatic Polypeptide (HPP)	615-10548-248-19	20	39±7	95±24	29±4	52±5
Pancreatic Glucagon (PG)	30K	20	135±8	147±16	119±12	118±16
Peptide Histidine-Methionine (PHM)	K210	10	26±4	28±2	21±4	36±3
Peptide YY (PYY)	80	20	117±11	86±5	99±8	110±15
Substance P (SP)	4892	15	17±3	<15±--	16±2	18±4
Vasoactive Intestinal Polypeptide (VIP)	4823	10	25±2	28±4	19±16	29±4

463.17 RELEASE OF SUBSTANCE P INTO THE SYNOVIAL FLUID, SUBDERMAL SPACE AND LYMPH FROM PERIPHERAL TERMINALS OF SMALL SENSORY AFFERENTS BY ANTIDROMIC STIMULATION. T.L. Yaksh*, J.E. Bailey* and G.J. Harty* (SPON: V.A. Lennon). Neurosurgical Research and GI Hormone Research Laboratory, Mayo Clinic, Rochester, MN 55905.

Substance P (sP) is contained in the peripheral terminals of small primary afferents. In the present studies we sought to determine whether these peripheral stores could be released into the extracellular space surrounding the nerve terminal. To accomplish these experiments, halothane (0.8%) anesthetized, artificially ventilated, cats were prepared with sciatic nerve electrodes placed at the ischeal notch and the nerve cut proximally to the spinal cord. To perfuse the extracellular spaces, three approaches were taken: 1) knee joint (KJ) perfusion by the insertion of inflow and outflow needles placed into the synovial fluid; 2) intradermal perfusion of a bleb (SB) formed on the dorsum of the depiliated paw with inflow and outflow needles; or 3) collection of spontaneously draining lymph (LYM) following catheterization with PE-10 of the saphenous lymph duct just below the politeal space. Perfusions were carried out with a modified Krebs solution at 50 µl/min using a two channel peristaltic pump for each site. Samples were assayed for sP using antibody #4892. This antibody cross reacts with sP_{1-11}/sP_{6-11} and sP_{1-11}/sP_{1-9} and it shows a progressive loss of cross reactivity following more extensive removal of either N or C terminal amino acids. In unstimulated samples, levels of sP were frequently undetectable in KJ/SB (<1-3 pg/ml). Stimulation of the sciatic nerve (30 V, 1.0 msec, 50 Hz, 1 sec on / 1 sec off) resulted in significant reversible increases in sP-like immunoreactivity at all sites. Addition of capsaicin (5×10^{-4}M) to the perfusate also resulted in significant increases in sP levels. The effects of sciatic nerve stimulation following capsaicin was diminished. In preliminary experiments, the addition of sufentanil (10^{-5}M) to the perfusate abrogated the evoked release of sP in the KJ and SB perfusates. These data suggest that the peripheral terminals of small primary afferents may possess a pharmacology comparable to that of the central terminals. (Supported by NIH grant NS-16541, TLY.)

Levels of sP-like Immunoreactivity in Effluent: $\bar{x} \pm$ SE (pg/ml)			
	KJ	SB	LYM
Control	3.2±0.3 (10)	1.9±0.1 (10)	26±5 (5)
Sciatic nerve	47±15* (6)	31±13* (6)	49±7* (4)
Capsaicin	59±14* (10)	49±8* (10)	-
Sciatic nerve post-capsaicin	7±5 (6)	10±8 (6)	-

( )Number of experiments; *P<0.01 as compared to pre-stimulation control.

463.18 ENDOGENOUS CLONIDINE-DISPLACING SUBSTANCE (CDS) FROM BOVINE BRAIN IS BIOLOGICALLY ACTIVE ON SMOOTH MUSCLE. *P. Ernsberger, D. Felsen*, M.P. Meeley, and D. J. Reis.* Div. of Neurobiology, Cornell U. Med. Coll., New York, NY 10021.

Clonidine-displacing substance (CDS) is a low-molecular-weight endogenous substance in brain (Atlas & Burstein, *Eur J Biochem* 144:287, 1984; Meeley et al., *Life Sci* 38:1119, 1986). We sought to determine whether CDS, like many neurotransmitters, is biologically active on smooth muscle. CDS was partially purified from bovine brain (Meeley et al., *op cit*). Smooth muscle preparations were suspended on force transducers with silk suture and superfused with oxygenated Krebs' buffer at 10 ml/min. Bolus applications of CDS had no effect on arterial (rat and rabbit aorta) or venous (dog and rabbit saphenous vein) vascular smooth muscle. The effects of CDS on rabbit jejunum and ileum were biphasic (contraction/relaxation) and variable. In contrast, CDS induced rapid, consistent, and dose-dependent contractions of the rat gastric fundus. In order to characterize the response to CDS, contractions were standardized to the peak tension elicited by prostaglandin E_2 (PGE_2; 200 pmol). One Unit of CDS (equivalent to the amount of brain extract required to inhibit ^{3}H-p-aminoclonidine binding by 50% under standard conditions) induced a contraction which was 82 ± 2% of that elicited by PGE_2. To determine whether CDS acted directly on fundic smooth muscle or on intrinsic neurons, fundic strips were continuously superfused with tetrodotoxin (TTX; 0.1 µM). The response to CDS was not blocked, but slightly facilitated (peak tension = 113 ± 1% of control). To determine whether the contractile response to CDS is mediated by Ca^{2+} channels, fundic strips were superfused with the Ca^{2+} channel blocker verapamil (1.0 µM). This treatment abolished the effect of CDS.

The rat fundic strip is responsive to a variety of endogenous compounds. The contractile activity of partially-purified CDS might therefore be due to contamination by known bioactive substances. To determine whether the biological action of CDS is unique, one of several agonists, or CDS, was applied as a bolus to fundic strips. Appropriate antagonists were infused at increasing concentrations until complete blockade of the response to an agonist was achieved. Tested singly, none of the antagonists blocked the contractile effect of CDS. The combined effect of blocking agents was tested using a cocktail containing: atropine, des-Arg^9-Leu^8-bradykinin, naloxone, phenoxybenzamine, promethazine, propranolol, and theophylline (all at 1.0 µM), methysergide, penicillamide^1-Val^4-Arg^8-vasopressin (0.5 µM), TTX (0.1 µM), and saralasin (30 nM). This cocktail of antagonists had no effect on the contractile response to 1 Unit of CDS (85 ± 23% of control), indicating that the action of CDS is not due to biogenic amines, adenosine, angiotensin, opiates, or vasopressin. Specific antagonists are not available for neurotensin and Substance P. However, cross-desensitization experiments showed that a constant infusion of agonist eliminated the effect of bolus doses of the same peptide, but had no effect on the contractile activity of CDS. In the presence of methysergide to block serotonin receptors, yohimbine (5 µM) inhibited the effect of CDS (73 ± 4% of control) but not PGE_2 (109 ± 4% of control). In conclusion, partially-purified CDS induces a rapid, dose-dependent contraction of the rat gastric fundus by a direct action on smooth muscle which is dependent upon Ca^{2+} channels. The contractile action of CDS appears distinct from known brain-gut transmitters, suggesting that CDS may be a unique substance.

463.19 ELECTROPHYSIOLOGICAL CHANGES INDUCED BY MELATONIN ON BRAIN NUCLEI OF THE RAT. C. Reyes-Vázquez, E.B. Naranjo-Rodríguez* and J.A. García-Segoviano*. Depto. de Farmacología, Facultad de Medicina. Universidad Nacional Autónoma de México.

Melatonin (MEL), the main hormone of the pineal gland, induces important effects, both in animals and humans. Such effects are modifications on sleep patterns, on thermoregulation, on locomotor activity and affective behavior between others. These effects suggest actions on central nervous system structures, mainly in limbic system. So far, we do not know if such actions are produced by the interaction between MEL and specific binding sites, or are mediated by other substances activated by MEL, because it has been shown that MEL is able to modify, both the production and the release of a great amount of hormones and neurotransmitters in several CNS sites. Obviously, a way to descriminate a possible direct action of MEL on brain cells, could be to analize the changes in the electrical activity of these cells, after MEL administration. According to this idea, we decided to record the effects induced by MEL sistemically administered on the multiunitary activity of several brain nuclei in unanesthetized freely-moving animals. Fourty two Wistar male rats (180-230 g) were implanted with semi-microelectrodes (60 µm) in the following structures: somatosensorial cortex (SSC), lateral hypothalamus (LH), medial thalamus (MT), amygdala (AM), mesencephalic reticular formation (MRF) and caudate nucleus (CN). Five days after surgery, we recorded the multiunitary activity using conventional techniques, 4 different structures were recorded simultaneously in each animal by session. The recorded multiunitary activity was analyzed on line, by means of a window discriminator, an analog-to-digital converter and a computer DEC 11/03. This computer calculated frequency and accumulative histograms. In the recording session and after a 45 min period of habituation, we recorded the control multiunitary activity during 10 min, after this period, one MEL dose (100, 200, 400, 500 or 1000 µg/kg) was applied i.p. and the recordings continued by 90 min more. Control inyections (1% ethanol) and sham inyections were made in most of the animals. From the 6 recorded structures, only MRF, LH and AM showed significant MEL effects. MRF showed a biphasic effect induced by MEL. Thus, frequency was increased with low doses and depressed with higher doses. However, in LH and AM only a decrease of the frequency of discharge was observed. These changes started 5 to 10 min after MEL administration and lasted for 25 to 30 min. The pharmacological characteristics of these MEL effects let us suggest that this hormone could exert direct actions on the electrophysiological properties of the cells from these tested structures.

464.1 EVIDENCE THAT AT LEAST TWO GENES ENCODE MONOAMINE OXIDASE
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Monoamine oxidase (MAO) is the enzyme primarily responsible for degradation of amine transmitters. Two forms of the enzyme termed MAO-A and MAO-B have been described. Both forms are thought to consist of two subunits, one of which bears a covalently bound flavin cofactor. These forms differ in apparent MW, peptide maps, substrate affinity, inhibitor sensitivity and distribution in the nervous system.

A synthetic oligonucleotide corresponding to known amino acid sequence of bovine MAO-B has been used to screen bovine adrenal medullary cDNA libraries. Two overlapping cDNA clones which span a 3.1 kb region have been isolated. Comparison of the cDNA-encoded amino acid sequence with those of peptides obtained by tryptic or endoproteinase lys-C digestion of bovine MAO-B show about 85% homology with scattered differences. These differences do not appear to be consistent with alternate splicing of a common precursor RNA. Northern blots hybridized with one of the cDNA clones which lies at the 5' end of the mRNA reveals a 3.7 kb poly A+ mRNA. The amount of this mRNA species in various tissues corresponds to levels of MAO-A enzyme activity. Human cDNA clones of MAO have also been isolated from placenta and liver libraries using the bovine cDNAs as probes. Preliminary DNA sequencing data suggest that there are at least two MAO mRNAs which arise from different genes in human.

Two segments of deduced amino acid sequence which are similar to conserved sequences in flavoproteins have been recognized. A segment near the presumed N-terminus corresponds to the putative adenine binding site and a segment near the FAD-attachment site in the C-terminal region corresponds to the putative contact site for the phosphate group of FMN.

These studies suggest that different genes encode MAO-A and MAO-B polypeptides and open the way for further understanding of structural and functional properties of these enzymes.

464.2 PURIFICATION OF THREE FORMS OF HUMAN PLATELET PHENOL SULFOTRANSFERASE.
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Phenol sulfotransferase (PST) catalyzes the transfer of a sulfonate moiety from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to a variety of catechol and phenolic acceptor molecules. Human platelets contain at least two functionally different forms of PST as characterized by their substrate specificities. The M species of PST is capable of sulfating phenolic and catechol monoamines such as dopamine and norepinephrine whereas the P form of the sulfotransferase selectively conjugates micromolar concentrations of phenol. Three distinct forms of PST from human platelets have been identified and characterized in our laboratory. These enzymes have been purified by ion exchange chromatography with DEAE cellulose followed by gel filtration with Sephacryl S-200 and affinity chromatography on a 3',5'-adenosine diphosphate agarose column. Two of the forms of PST (M_I and M_{II}) were capable of sulfating dopamine while the third form which was separated by ion exchange chromatography was incapable of sulfating this catecholamine and therefore resembled the P species of PST. The two M species of PST were copurified by the procedures described above and had a final specific activity of 1 x 10^5 pmoles/min/mg protein using dopamine as substrate. The isolated P-PST had a specific activity of 1.3 x 10^5 pmoles/min/mg protein using phenol as substrate. The combined M forms of PST were purified a minimum of 1500 fold over the 100,000 x g supernatant solution while P-PST was purified at least 3200 fold. The native molecular weight determined from Sephacryl S-200 gel filtration chromatography was estimated to be approximately 66,000 for all three forms of PST. Based on SDS-PAGE analysis, the molecular weight of the subunit for P-PST was approximately 30,000 whereas the molecular weights for the M_I and M_{II} subunits were 32,000 and 34,000, respectively. Preliminary data suggest that M_I PST may also have a high affinity for phenol. These data suggest that both M and P PST may exist as dimers in their functionally active form in vivo. Antibodies to all three forms of PST have been raised in rabbits. Based on immunoblot analysis, the two M-specific antibodies failed to cross react with the P species of PST whereas the P-specific antibody showed no cross reactivity with either of the two M forms of the sulfotransferase.

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464.3 LOCATION OF MONOAMINE OXIDASE (MAO) TYPES A AND B IN THE ENTERIC NERVOUS SYSTEM OF THE GUINEA PIG SMALL INTESTINE.
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Monoamines found in the enteric nervous system (ENS) include serotonin (5-HT) and norepinephrine (NE). 5-HT is present in intrinsic neurons of the myenteric plexus, which also send axons to the submucosal plexus. NE is located in sympathetic axons, which originate from extrinsic prevertebral ganglia. In the human and rat CNS, the noradrenergic neurons of the locus ceruleus primarily contain MAO-A, while the serotoninergic neurons of the raphe nuclei contain MAO-B. The ENS has been shown to be rich in MAO, but the type specificity of enteric neural MAO has not previously been determined. In order to understand enteric monoaminergic function it is important to know the type and location of MAO in the ENS. We therefore studied enteric MAO both immunocytochemically, with MAO-B-specific antisera, and histochemically, with a coupled peroxidation method following preincubation with specific inhibitors (clorgyline, 0.01-10.0 μM for MAO-A and deprenyl, 0.1-10.0 μM for MAO-B). In order to visualize putative transmitters simultaneously with MAO-B, double immunocytochemistry was done using primary antisera from different species and contrastingly labeled species-specific secondary antisera. In addition, immunocytochemical localization of putative transmitters was done simultaneously with the type-specific histochemical demonstration of MAO activity. For this purpose immunoreactivity was visualized using biotinylated secondary antibodies and avidin-alkaline phosphatase with a red reaction product. MAO-B immunoreactivity was found in intrinsic neurons and neurites in both myenteric and submucosal plexuses. Histochemically, MAO activity in intrinsic neurons was inhibited by deprenyl (0.1 μM), but not by clorgyline (0.1 μM), confirming that MAO-B is the primary type of MAO found in the intrinsic elements of the ENS. In contrast, MAO-A activity (clorgyline, but not deprenyl sensitive) was abundant in varicose sympathetic terminal axons, which degenerated following administration of 6-hydroxydopamine. MAO-B could not be demonstrated immunocytochemically or histochemically in sympathetic axons or in the enteric glia, nor could signficant MAO-B be shown to co-exist with 5-HT immunoreactivity in neuronal cell bodies. MAO-B did co-localize in neurons containing immunoreactive neuropeptides. In the submucosa, where about half the neurons contained MAO-B, co-localization was found with neuropeptide Y (NPY) and calcitonin gene related peptide (CGRP), but not substance P or vasoactive intestinal polypeptide (VIP). About 10 % of myenteric neurons were intensely reactive for MAO-B. Many of these contained CGRP. Other myenteric neurons had only moderate MAO activity. Since MAO-B activity in cell bodies was greatly increased following administration of colchicine (5 mg/kg; 4-5 hrs.), MAO-B appears to be translocated proximo-distally by axonal transport. It is concluded that a subset of intrinsic enteric neurons contain, mainly MAO-B, although the possibility that some may also contain small amounts of MAO-A has not been excluded. Enteric sympathetic nerves contain MAO-A, but not MAO-B. The type of MAO, if any, in enteric serotoninergic neurons is not clear because little MAO-B is found in the cell bodies of these neurons. MAO-B is found in many enteric neurons that are not monoaminergic, but peptidergic. The role of MAO in these neurons remains to be determined. Supported by NIH grants NS12969, NS15547, and HD18592.

464.4 ULTRASTRUCTURAL LOCALIZATION AND LIGHT MICROSCOPIC SURVEY OF MAO-B CONTAINING CELLS IN CAT CENTRAL NERVOUS SYSTEM.
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Little is known concerning the cellular and regional ultrastructural localizations of monoamine oxidase (MAO) subtypes in the brain. Most studies, until very recently, have relied upon identifying these enzymes in brain homogenates rather than in intact tissues. Therefore, the present study was performed to examine the cellular, regional and ultrastructural localization of MAO-B in the intact cat brain. Cats were anesthetized and perfused transcardially with either 4% paraformaldehyde (PF) in phosphate (pH 6.0) and borate (pH 11.0) buffers (for light microscopy) or with 4% PF in phosphate buffer (pH 7.4) plus 0.1% to 0.2% glutaraldehyde [for electronmicroscopy (EM)]. All tissue was vibratome cut (30 to 50 μm) and processed for avidin-biotin immunohistochemistry using DAB as a chromogen. Tissue punches from glutaraldehyde-fixed sections were embedded for EM. MAO-B was localized to neural and glial elements in cat brain. While MAO-B positive neurons were found in the hypothalamus, raphe system, locus coeruleus and dorsal pontine tegmentum, no labeled neurons were found in ventral mesencephalic dopamine regions. Labeled glial cells were found in almost all areas of brain. There was also a significantly greater density of MAO-B glial cells in the substantia nigra (SN) than in other nearby catecholamine cell groups. At the ultrastructural level, MAO-B was found in processes of astrocytes adjacent to terminals containing synaptic vesicles in the substantia nigra. MAO-B was also found in astrocytic footplates adjacent to capillary endothelium. In dorsal raphe and lateral hypothalamic nuclei, MAO-B was observed in neuronal somata with reaction product in cytoplasm and often associated with mitochondria. The localization of MAO-B in certain neural elements (i.e., neuronal somata, dendrites, boutons and glial cells) in various brain regions should help to clarify the role of this enzyme in influencing neurotransmitter function. Supported in part by grants USHHS MH41645 and DE7282.

464.5 INTERACTIONS OF TRICYCLIC ANTIDEPRESSANT DRUGS WITH HUMAN AND RAT MONOAMINE OXIDASE TYPE B. A.A. Reid*, J.L. Hill* and D.L. Murphy*. (Spon: H. Holloway). Lab of Clinical Science, National Institute of Mental Health, NIH 10/3D41, Bethesda, MD 20892.

Monoamine oxidase (MAO) exists as two isozymes, type A and B, which are both found in human and rat brain, while only MAO type B exists in human platelets. Tricyclic antidepressant drugs are primarily classed as monoamine uptake inhibitors, but they also act as weak reversible inhibitors of MAO in vitro, with a greater affinity for MAO type B. They inhibit the deamination of phenylethylamine and benzylamine, two MAO type B substrates, non-competitively and competitively, respectively.

We have further investigated the effects of tricyclic antidepressant drugs on the deamination of phenylethylamine and benzylamine by MAO type B, in vitro in human brain cortex, human platelet and rat brain preparations. These drugs inhibited MAO activity as expected, but, in addition, in human cortex and human platelet preparations a biphasic inhibitory response was observed when benzylamine was used as the substrate with 10^{-3} M imipramine and amitriptyline, two tertiary amine tricyclics, and an activation occurred with 10^{-3} M clomipramine, another tertiary amine tricyclic. The biphasic inhibitory pattern or activation was not reproduced when the secondary amine tricyclics, desipramine and desmethylclomipramine, or the secondary amine bicyclic antidepressant drug, fluoxetine, were studied or when phenylethylamine was utilized as the substrate or when rat brain was used.

Of the tricyclics which exhibited normal inhibition patterns, the same rank order of inhibition occurred among the drugs in both human tissues but not in the rat brain when phenylethylamine was used as the substrate, while with benzylamine the same inhibitory relationship was noted across all tissues. In general, the drugs inhibited the deamination of both substrates in the various tissues with the following potencies: rat brain > human platelet > human cortex ($p<0.05$). Furthermore, each tricyclic was a more potent inhibitor of phenylethylamine than benzylamine deamination ($p<0.01$), except for desmethylclomipramine which inhibited both substrates similarly. On the other hand, fluoxetine inhibited benzylamine deamination to a greater extent than phenylethylamine in human cortex ($p<0.01$) and tended to do so also in human platelet and rat brain.

These tricyclic-MAO interactional data suggest that rat and human MAO type B are not functionally identical, that secondary and tertiary amine tricyclics interact differently with MAO type B, that fluoxetine, a bicyclic antidepressant, interacts differently from the tricyclics, and also support other data that phenylethylamine and benzylamine are deaminated by different mechanisms.

464.6 COCAINE AND REGIONAL MONOAMINE UTILIZATION IN MOUSE BRAIN. M.G. Hadfield and C. Milio*, Neurochemistry Research Laboratory, Section on Neuropathology, Department of Pathology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA. 23298

Cocaine inhibits the uptake and enhances the release of central monoamines. This prolonges the activity of neurotransmitters at the synapse and may account for much of cocaine's action on the central nervous system. The present study was undertaken to see if cocaine produces selective changes in utilization of norepinephrine, dopamine and/or serotonin, depending on the brain region and neurotransmitter system examined.

In the present study, adult male ICR mice were given saline or 10 mg/kg cocaine i.p. 1/2 hr. before removal of the brains which were quickly frozen in liquid nitrogen. The olfactory bulbs (OB), prefrontal cortex (PC), striatum (ST), amaydaloid nuclei (AMY), hypothalamus (HT) and thalamus (TH) were dissected and extracted in acetate buffer containing ascorbate oxidase for HPLC analysis of monoamines and metabolites (Hadfield et al., J.Chromatogr. 369:449-453, 1986 and J. Liquid Chromatgr. In Press). Regional brain levels of the following compounds were measured: NE and its metabolite, 3,4-dimethoxyphenylglycol (MHPG), DA and its metabolites, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3MT) as well as serotonin (5HT) and its metabolite, 5-hydroxyindoleacetic acid (5HIAA). Ratios of metabolite levels to those of their parent neurotransmitters were calculated in order to determine utilization of neurotransmitters. The following significant decreases were noted.

	OB	PC	ST	AMY	HT	TH
MHPG/NE				↓	↓	
DOPAC/DA		↓	↓	↓		
HVA/DA		↓		↓	↓	
3MT/DA		↓				
THIAA/5HT				↓	↓	↓

In all instances there was decreased utilization of neurotransmitter as indicated by the decrease in the metabolite/neurotransmitter ratios. However, the pattern was different depending on the neurotransmitter and the tissue. The noradrenergic system was altered in the AMY and HT while the serotonergic system was altered in the AMY, HT and TH. The DOPAC/DA ratios were altered in the PC. ST and AMY while the HVA/DA ratios were affected in the PC, AMY and HT. The 3MT/DA ratio was altered only in the PC. The greatest effects were noted in the prefrontal cortex, amygdala and hypothalamus, areas which have been related to various behavioral and emotional states. The direction of the changes will be discussed in terms of cocaine's mechanisms of action on the central nervous system and the pattern of changes to relevant clinical effects produced by cocaine.

464.7 EFFECTS OF SELECTIVE MAO-A INHIBITORS ON BRAIN BIOCHEMISTRY AND RESPONSES IN ANIMAL MODELS OF DEPRESSION. H.L. White, R.M. Norton*, F.E. Soroko* and B.R. Cooper. Department of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709.

BW A616U and BW A70C are both reversible and selective inhibitors of monoamine oxidase-A (MAO-A). However, although BW A70C was at least 4-fold more potent than BW A616U as an MAO-A inhibitor, both in vitro and ex vivo, it was much less active as an antagonist of sedation in the tetrabenazine and Porsolt models of depression. The purpose of this study was to determine whether changes in brain levels of specific substrates or products of MAO could be correlated with behavioral responses in the animal models.

Inhibitors were administered orally to Sprague-Dawley male rats at various times before sacrifice. Brains were rapidly removed and immediately frozen to await biochemical testing. Biogenic amines and their metabolites were assayed in brain extracts using HPLC techniques. MAO-A and MAO-B were estimated in brain homogenates using [^{3}H] serotonin and [^{14}C] B-phenethylamine as substrates in a double-label radiochemical assay.

After 50 mg/kg p.o., both inhibitors produced similar elevations of major biogenic amines. Brain serotonin reached 134-142% of control, and norepinephrine, 120-127% of control, with maxima between 2 and 4 hr after dosing. Smaller, but significant elevations of dopamine also occurred (110-120% of control). MAO-A was inhibited by at least 80%, and no inhibition of MAO-B was observed with either compound. Other experiments showed similar patterns of tissue distribution for the two inhibitors.

The major difference between the two compounds was in levels of certain products of MAO. For example, DOPAC, a dopamine metabolite, was reduced to approximately 30% of control level after BW A616U administration, but was about 60% of control after BW A70C. Although the latter compound was the more potent MAO-A inhibitor, it produced weak and inconsistent results in the animal models.

We conclude that effects in the tetrabenazine and Porsolt models of depression by these MAO-A inhibitors are not simply related to elevated levels of brain serotonin, norepinephrine, or dopamine, but may involve more complicated interactions with brain monoamine systems.

464.8 EFFECTS OF ADRENALECTOMY ON THE 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA)-INDUCED DECREASE OF TRYPTOPHAN HYDROXYLASE ACTIVITY IN THE FRONTAL CORTEX AND HIPPOCAMPUS. M. Johnson*, L.G. Bush*, D.M. Stone*, G.R. Hanson and J.W. Gibb (SPON: F. Matsuo). Dept. Pharmacol. and Toxicol., University of Utah, Salt Lake City, UT 84112.

The administration of a single high dose of the psychoactive amphetamine analog, 3,4-methylenedioxymethamphetamine (MDMA), decreases brain 5-HT concentrations for up to 7 days (Schmidt et al., 1986). The changes occurring 7 days after drug treatment are accompanied with a decrease in synaptosomal 5-HT uptake, suggesting that neuronal damage has occurred (Schmidt, 1987). Several reports indicate that glucocorticoids affect the neuronal degeneration observed during aging or induced by the application of 3-acetylpyridine (Sapolsky, 1986). The purpose of this study was to determine whether glucocorticoids could be involved in the decrease of rat brain TPH activity after the administration of MDMA.

Male Sprague-Dawley rats were anesthetized with ketamine (175 mg/kg) and the adrenals were removed by dorsal incision. The animals were housed 3 per cage and the adrenalectomized rats received 0.9% saline in lieu of water. After 5 days, the rats were injected with 10 or 20 mg/kg MDMA (s.c.) and sacrificed 7 days later. TPH activity was determined according to Ichiyama et al (1970) and Sitaram and Lees (1978).

TPH activity in the frontal cortex remained unaffected 7 days after administration of 10 mg/kg of MDMA while the enzyme activity was decreased to 51% of control when a 20 mg/kg dose was used. Adrenalectomy had no effect on the MDMA-induced decrease of enzymatic activity in the frontal cortex. However, MDMA decreased the hippocampal TPH activity to 78% and 49% of control after a 10 and 20 mg/kg dose, respectively. Adrenalectomy prevented the decrease in TPH activity observed with the 10 mg/kg dose, and the decrease by the higher dose was significantly attenuated to 68% of control. These results suggest that glucocorticoids may contribute to the MDMA-induced long-term decrease in TPH activity observed in the hippocampus after a single dose; this response appears to occur in selected brain areas. (Supported by USPHS grants DA 00869, DA 04222 and Janssen Pharmaceutica Inc.)

464.9 THE DETERMINATION OF FREE HOMOVANILLIC ACID (HVA), (3-METHOXY-4-HYDROXYPHENYL) ETHYLENE GLYCOL (MHPG), AND VANILMANDELIC ACID (VMA) IN HUMAN SALIVA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH DUAL-ELECTRODE COULOMETRIC ELECTROCHEMICAL DETECTION. C.J. Drebing*, G.A. Gerhardt, C. Stephen* and R. Freedman, Depts. of Psychiatry and Pharmacology, Univ. of Colorado Hlth. Sci. Ctr., Denver, CO 80262, and Medical Research Service, Veterans Administration Medical Center, Denver, CO 80220.

Many studies to date have demonstrated the usefulness of measuring catecholamine metabolite levels found in biological fluids in studies of psychiatric disorders such as mania and schizophrenia. Several of these studies have indicated that differences in levels of these compounds are due to factors such as diet, exercise and diurnal variation. Our laboratory has been interested in levels of the dopamine metabolite HVA, as well as the noradrenergic metabolites MHPG and VMA, and whether or not these compounds could be measured in saliva. In addition, we wanted to know if changes in saliva levels of the catecholamine metabolites would directly correlate with levels seen in plasma. Saliva is a bodily fluid that can be obtained in an easy and noninvasive manner, providing repeated measures of catecholamine metabolite levels that would be especially useful in clinical studies involving children.

The determination of the methoxylated catecholamine metabolites entails extraction of the compounds from saliva with ethyl acetate, following acidification and addition of sodium chloride. The ethyl acetate fractions are combined and evaporated to dryness. The resulting residues are resuspended in pH 3.0 buffer and injected into a high performance liquid chromatography system coupled with coulometric electrochemical detection. The methoxylated compounds are separated and quantitated using a gradient elution procedure similar to the method that we have recently reported (Gerhardt, G.A., Drebing, C.J. and Freedman, R., Anal. Chem., 58: 2879, 1986).

Initial results support that levels of salivary catecholamine metabolites correlate with plasma levels. Correlations range from 0.95 for HVA to 0.65 and 0.57 for MHPG and VMA respectively. (Supported by USPHS grants MH-38321 and DA-02429, and VA Medical Research Service.)

464.10 DETERMINATION OF FREE AND BOUND METABOLITES OF NOREPINEPHRINE IN RAT BRAIN BY HPLC WITH SERIAL OXIDATIVE-REDUCTIVE ELECTROCHEMICAL DETECTION P. H. Desan, G. A. Gerhardt, W. Woodmansee*, T. Smock. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309 and Dept. of Psychiatry, Univ. of Colorado Health Sci. Ctr., Denver, CO 80262

High performance liquid chromatography coupled with electrochemical detection is an efficient means for the concurrent quantification of monoamines and their metabolites in brain. Unfortunately, measurement of the principal metabolites of norepinephrine, dihydroxyphenyl glycol (DHPG) and 3-methoxy-4-hydroxyphenyl glycol (MHPG), is difficult because they are extensively sulfated in most rat brain regions. In addition, other electroactive species coelute with these compounds, and interfere with the measurement of even their free quantities. We describe here methods for the measurement of free and total DHPG and MHPG in rat brain using serial coulometric detection.

Frozen samples of anterior cortex, hippocampus and cerebellum were sonicated in 0.1M acetate pH 5 buffer and centrifuged at 17000g for 20'. The supernatants were injected directly on a Beckman ODS C18 reverse phase column (15cm x 4.6mm, 5μ packing). A pH 4.00 citrate-acetate:octane sulfonic acid:EDTA mobile phase was used at a flow rate of 1.6 ml/min. Column outflow was passed serially through three coulometric detectors (5020 Guard Cell and 5011 Dual Cell, with Model 5100A Coulochem Controller, ESA, Inc.): the first detector serves to oxidize compounds of interest at +.35 - +.50v, the second to screen out easily reduced interferents at 0 - -.10v, and the third for peak quantification in the reducing mode at -.35 - -.40v. With careful adjustment of cell potentials, DHPG and MHPG can be accurately measured at the third detector. This detector configuration permits the simultaneous determination of norepinephrine, dopamine and its metabolites DOPAC and HVA, serotonin and its metabolites 5HIAA and 5OH tryptophol, tyrosine, tryptophan and uric acid. Finally, following treatment with sulfatase (S3009, Sigma, 4 mg/ml sample volume) for 12 hours at 35C a sample aliquot can be injected directly for determination of free plus sulfated DHPG and MHPG.

Thus, the monoamine neurotransmitters, their precursor amino acids and their metabolites, including the free and bound metabolites of norepinephrine, can be determined in a single brain sample.

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464.11 REGIONAL ANALYSIS OF RAT BRAIN DOPAMINE AND SEROTONIN METABOLISM AFTER AN ACUTE LOW DOSE OF ETHANOL. Steven A. Signs*[1], Bryan K. Yamamoto[2] and Martin D. Schechter[2] (SPON: M. Mangiapane). [1]Dept. of Pharmacology, Univ. of Rochester Med. Ctr., Rochester, NY, 14642 and [2]Dept. of Pharmacology, NEOU College of Medicine, Rootstown, Ohio 44272.

Research on the effect of high doses of ethanol (greater than 2 g/kg) on rat brain dopamine and serotonin metabolism has resulted in variable results ranging from increases or decreases in metabolism, to no discernable change. The dose of ethanol in these studies (> 2 g/kg) elicits a sedative-hypnotic type reaction in laboratory animals which may not truly represent the reinforcing component thought to be responsible for ethanol abuse. Therefore we have chosen a low dose of ethanol (0.6 g/kg IP), shown in our lab to reliably control discriminative performance in rats, to examine the effect of an acute low dose ethanol administration on dopamine and serotonin metabolism in discrete areas of the rat brain.

Eighteen experimentally-naive male S-D rats weighing 250-300g were food deprived for 24 hrs prior to experimentation. Six animals were injected with water (5 ml/kg IP) as controls and 12 animals received an injection of 600 mg/kg ethanol IP (10% V/V in distilled H_2O). After 10 min, 6 control and 6 ethanol-treated animals were sacrificed by decapitation and the brains frozen on dry ice. Thirty min post-injection, the remaining 6 animals were sacrificed. Brain tissue specimens were harvested from the nucleus accumbens (NA), anterior caudate-putamen (CP), dorsal raphe (DR), and substantia nigra (SN) by coronal cryosection and punch biopsy microdissection. The simultaneous analysis of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) was undertaken as follows. Brain tissue specimens were homogenized in cold 0.1 N perchloric acid and briefly centrifuged at 10,000 x g. The resulting supernatant was chromatographed on Biophase ODS 5 μm stationary phase using a mobile phase containing 0.15 M monochloroacetic acid (pH 3.0), 200 mg/l OSS, 3.6% acetonitrile, 1.8% tetrahydrofuran. Biogenic amines and metabolites were detected electrochemically at an applied potential of + 0.8 V.

Ten minutes after ethanol administration, no changes could be detected for any compound tested nor in any brain area. However, after 30 min, the content of serotonin and its major metabolite, 5-HIAA, increased significantly ($p < 0.05$) in nucleus accumbens. Total tissue content of serotonin in nucleus accumbens increased 36% whereas 5-HIAA was elevated 25%. These results indicate that an acute, low dose of ethanol results in an increase in the metabolic activity of serotonergic terminals innervating the nucleus accumbens in rats.

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464.12 INTRACEREBRAL DIALYSIS: 3-METHOXYTYRAMINE (3-MT) MEASUREMENT AS AN INDEX OF DOPAMINE (DA) RELEASE. H. S. Kim, M. R. Marien and P. L. Wood, Neuroscience Research, Research Dept., Pharmaceutical Division, Ciba-Geigy Corp., Summit, N.J. 07901.

Intracerebral dialysis was used to study in vivo DA release in the striatum, with a special emphasis on changes in concentrations of the extracellular DA metabolite, 3-MT. Both basal and d-amphetamine-stimulated levels of DA, 3-MT, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured in sodium pentobarbital-anesthetized rats. Dialysates were analyzed by gas chromatography-mass fragmentography in the negative chemical ionization mode.

The baseline levels of DA and its metabolites were stable over a 2 hour period (fmol/ 10 min collection: DA = 71 ± 14; 3-MT = 23 ± 1.7; DOPAC = 17,700 ± 400; HVA = 10,200 ± 200). Amphetamine (5 mg/kg ip) caused a rapid increase (1500 %) in DA levels within 20 min after administration and this effect was followed by increases in 3-MT levels (1300 %) 15-30 min later. Under both basal and d-amphetamine-stimulated conditions, the 3-MT pool was maintained at approximately 30 % of the extracellular DA pool. DOPAC levels showed a slow decline while HVA levels were not significantly altered by amphetamine.

Our data suggest that under the present experimental conditions, approximately 30 % of the extracellular DA pool is metabolized to 3-MT. This study supports the hypothesis that 3-MT is an accurate index of synaptic DA release.

464.13 THE EFFECT OF BUSPIRONE AND ITS METABOLITE ON SEROTONIN, DOPAMINE AND NOREPINEPHRINE TURNOVER IN RAT BRAIN. Kenneth W. Perry* and Ray W. Fuller. Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285.

Buspirone is an anxiolytic drug which is rapidly metabolized to 1-(2-pyrimidinyl)piperazine (1-PP) [Caccia et al., Pharmacology 33:46-51 (1986)]. Since the metabolite was present in brain at a concentration higher than that of buspirone, the possibility of it playing a role in the therapeutic or other actions of buspirone has been considered. Our purpose in the studies reported here was to compare the neurochemical changes produced in brain by buspirone and by 1-PP.

Buspirone caused a dose-related decrease in hypothalamic 5-hydroxyindoleacetic acid (5HIAA) concentration at doses from 1-10 mg/kg s.c. in rats. At a dose of 3 mg/kg the decrease was greatest at 1 hour (-35%), with a rapid return to near control levels at 4 hours. The decrease in 5HIAA was apparently due to a reduced turnover of serotonin since the accumulation of 5-hydroxytrytophan after decarboxylase inhibition by NSD 1015 was suppressed at doses of buspirone from 0.3 to 3 mg/kg s.c. Buspirone also increased the concentrations of the dopamine metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid measured in whole brain and in striatum, and increased the accumulation of DOPA in striatum after decarboxylase inhibition. The dose response and time course were similar to those described for 5HIAA. Buspirone antagonized the pergolide-induced decrease in striatal DOPA accumulation after decarboxylase inhibition in rats treated with 4-butyrolactone, indicating that buspirone blocks dopamine autoreceptors.

The buspirone metabolite, 1-PP, did not affect 5HIAA and had little effect on the dopamine metabolites over the same dose range.

Both buspirone and 1-PP, at doses from 0.3 to 10 mg/kg s.c., increased concentrations of the norepinephrine metabolite, MHPG-sulfate, in the three brain regions examined (hypothalamus, brain stem and midbrain). At a 3 mg/kg dose the increase reached a maximum at 2 hours in the hypothalamus with the effect of 1-PP (+76%) being slightly greater than that of buspirone (+60%). In both cases MHPG-sulfate levels had returned to normal by 8 hours. The increased norepinephrine turnover after buspirone might be due to formation of 1-PP.

The action of 1-PP may be mainly blockade of alpha-2 receptors [Caccia et al., above], whereas buspirone alters serotonin and dopamine metabolism, probably by activation of 5HT1A receptors [Peroutka, Biol. Psychiat. 20: 971-979 (1985)] and blockade of D2 autoreceptors [McMillen and Mattiace, J. Neural Trans. 57:225-265 (1983)], respectively.

464.14 EFFECTS OF HYPOXIA ON CATECHOLAMINE METABOLISM IN RABBIT SUPERIOR CERVICAL GANGLION. B. Dinger, G.-F. Cheng* and S.J. Fidone. Dept. Physiol., School of Medicine, Univ. Utah, Salt Lake City, Utah 84108.

In previous studies we demonstrated that hypoxia reduces the content of substance P (SP) and increases glucose utilization in superior cervical ganglia (SCG) which were either chronically decentralized or exposed to reduced O_2 *in vitro* (Dinger *et al.*, Neurosci. Abstr. 12: 888, 1986). Parallel studies with the arterial chemosensory tissue of the carotid body revealed similar changes in SP content and glucose consumption, while experiments with nodose sensory ganglia showed that hypoxia did not alter SP levels or intermediary metabolism. These findings suggest that the SCG contains specific internal chemosensory mechanisms similar to those of the carotid body. In the present study, we have evaluated changes in catecholamine (CA) metabolism induced in the SCG by hypoxia.

Desheathed SCG were preincubated for 40 min in Tyrode's solution equilibrated with 100% O_2 (37°C). The ganglia were transferred to vials containing media equilibrated with either 100% O_2, 40% O_2 or 20% O_2, where superfusion continued for 10 min (37°C). Incubation was terminated in 0.2N perchloric acid (PCA). The effects of hypoxia on CA metabolism were also studied in slices of rabbit corpus striata. PCA extracted CAs were separated and quantified using reverse phase HPLC with electrochemical detection.

The level of dihydroxyphenylalanine (DOPA) was low in ganglia incubated in 100% O_2 media (0.09 ng/mg of tissue). DOPA content increased 7.3x and 11.5x following exposure to 40% O_2- and 20% O_2-media, respectively. Likewise, hypoxia elevated the dopamine (DA) content of SCG by 41% ($p<0.05$) and 53% ($p<0.025$) in 40% O_2- and 20% O_2-media. In contrast, the norepinephrine (NE) content of the SCG was unchanged by hypoxia, and similar exposure of corpus striata did not alter the levels of CAs. Preliminary studies of CA release have revealed constant low levels of NE and DA in the superfusion media, and that the efflux of 3,4-dihydroxyphenylacetic acid (DOPAC, principle DA catabolite) tends to increase following hypoxia; thus, the elevated DA content induced by hypoxia cannot be attributed to decreased release.

The data indicate that hypoxia activates a select pool of tyrosine hydroxylase (TH), which terminates CA synthesis at the level of DA. Previous studies in our laboratory demonstrated that hypoxia increases DA synthesis and release from the carotid body, whose type I parenchymal cells, like the small intensely fluorescent (SIF) cells of the SCG, contain concentrated stores of DA. The finding that hypoxia alters DA metabolism in the SCG may have important consequences for sympathetic neurotransmission during stress. Supported by USPHS grants NS12636, NS07938.

464.15 ACUTE AND CHRONIC EFFECTS OF MDMA ON RAT MONOAMINE METABOLISM. R.T. Matthews, T.H. Champney and T.P. Friedlander*. Dept. of Med. Anatomy, Texas A&M University, College Station, TX 77843.

3,4-methylenedioxymethamphetamine (MDMA) is an amphetamine analog which has become a popular drug of abuse. Several laboratories have now shown that MDMA is toxic to serotonergic (5HT) neurons in rats and results in long-term loss of 5HT nerve terminals. In addition, there appears to be an acute depression in 5HT turnover and synthesis which may be independent of the more long-lasting toxicity. MDMA has little effect on the nigrostriatal or hypothalamic dopamine (DA) systems or on norepinephrine neurons. Additional experiments were undertaken to assess the effects of acute and chronic treatments with MDMA on monoamine metabolism in several unexplored brain regions and in the pineal of male albino rats. Standard HPLC separation with electrochemical detection was used for monoamine quantitation. In acute experiments, MDMA had a biphasic effect on the concentration of 5HT and the major 5HT metabolite, 5-hydroxyindole acetic acid (5HIAA). Fifteen to 30 minutes after i.p. injection of 10 mg/kg or more of MDMA, 5HT and 5HIAA were increased in the medial prefrontal cortex (MPC, 36%), striatum (35%), hypothalamus (23%) and caudal brainstem (pons and medulla, 30%). This was followed by a depression of 5HT and 5HIAA which was maximal at 2 hrs and began to recover at 6 hrs. This decrease was relatively more selective for 5HT nerve terminal areas than for 5HT neuron soma areas (caudal brainstem) and was dose-dependent (3-30 mg/kg). In the pineal, 5HT content was not affected at any time or dose, but 5HIAA transiently decreased by 42% (10 mg/kg MDMA, 1-2 hrs). MDMA (1-30 mg/kg, 2 hrs) slightly but non-significantly increased tryptophan concentrations in MPC and caudal brainstem. MDMA (10 mg/kg, 0.5-2 hrs) increased DA concentrations in the striatum by 33% and in the MPC by 30%. Concentrations of the DA metabolite, dihydroxyphenylacetic acid (DOPAC), were increased in the MPC (47%) but were decreased in the striatum (34%). In chronic experiments (10 or 20 mg/kg, s.c., every 12 hrs for 4 days, 2 weeks recovery), 5HT and 5HIAA concentrations were depressed in all areas, except the pineal. Tryptophan, DA and DOPAC concentrations were not affected in any area at either dose. In summary, these data suggest that 1) acutely, MDMA treatment has a small but significant effect on DA metabolism and the effect may be different in A9 (striatum) compared to A10 (MPC) DA terminal regions, 2) reduction in 5HT turnover and content after acute or chronic MDMA treatment are not caused by reduced availability of tryptophan and 3) 5HT nerve terminals appear to be more vulnerable to the acute and chronic effects of MDMA than are 5HT neuron soma or 5HT-containing neuroendocrine cells of the pineal.

464.16 3-HYDROXYANTHRANILIC ACID OXYGENASE IN HUMAN BLOOD PLATELETS. E. Okuno, A. Choe and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

The activity of 3-hydroxyanthranilic acid oxygenase (3HAO), the biosynthetic enzyme of the excitotoxic brain metabolite quinolinic acid, is substantially increased in the brains of Huntington's disease victims (Schwarcz et al., this meeting). To date, 3HAO measurements in blood cells, which may serve as markers for the status of the cerebral enzyme, have not been performed. We have now examined and report the presence and properties of 3HAO in human platelets.

Blood (10 ml) of human volunteers was collected in Venoject® tubes and platelets isolated according to established procedures. The platelets were resuspended in 1 - 2 ml isotonic sucrose, sonicated and preheated (1 min, 55°C) before use in the assay. Optimal conditions for the 3HAO assay, including linearity with protein amount and incubation time, were established in pilot experiments. Routinely, 50 µl of the homogenized platelets were incubated for 30 minutes at 37°C in 0.5 ml 0.1 mM MES buffer, pH 6.5, containing 6,000 dpm (2.4 µM) ^{14}C-3-hydroxyanthranilic acid and 3 mM $FeSO_4$. Blank tubes received boiled platelets. After the incubation, the assay mixture was centrifuged (10000 x g, 10 min) and the supernatant applied to a Dowex AG x 50 cation exchange resin. ^{14}C-quinolinic acid was eluted with 1 ml water and its radioactivity determined by liquid scintillation spectrometry.

3HAO activity was present in the platelets of all five normal individuals (2 males, 3 females) used in the study. Identity of the enzymatic product quinolinic acid was verified radioenzymatically (Anal. Biochem. 158, 98, 1986) in experiments using non-radioactive 3-hydroxyanthranilic acid in the assay. The activity of platelet 3HAO was approximately 6 times lower than that in human caudate nucleus, and was reasonably consistent when assessed in intra- and interpersonal test-retest experiments. No loss of activity occurred when the homogenate was frozen at -80°C. Millimolar concentrations of glutamate, aspartate, kainate, quinolinate and kynurenate in the assay did not interfere with 3HAO activity. Kinetic analysis revealed a K_m of 4.8 µM for the substrate 3-hydroxyanthranilic acid and a V_{max} value of 1.1 nmol/h/mg protein (N = 4).

The properties of platelet 3HAO suggest its usefulness as an easily accessible marker for QUIN biosynthesis in humans.

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464.17 SODIUM-DEPENDENT UPTAKE OF L-KYNURENINE INTO SLICES FROM RAT BRAIN. C. Speciale[1], W. A. Turski[1], N. Brookes[2] and R. Schwarcz[1]. [1]Maryland Psychiatric Research Center, Baltimore, MD 21228 and [2] Dept. Pharmacology, Univ. of Maryland School Medicine, Baltimore, MD 21201.

L-kynurenine (KYN) is the bioprecursor of both the neuroinhibitory compound kynurenic acid and the neuroexcitatory and excitotoxic brain metabolite quinolinic acid. KYN is known to be able to enter the brain from the periphery (Neurochem. Res. 5, 223, 1980) but the process responsible for its subsequent transport into brain cells has as yet not been directly examined. We report here the presence, in rat brain tissue, of specific uptake mechanisms for KYN.

In routine experiments, two 500 μm thick slices from adult rat striatum were incubated for one hour at 37°C in an oxygenated culture well containing 1 ml Krebs-Ringer buffer (pH 7.4) and 800 nM (0.2 μCi) ^{3}H-KYN (specific activity 8.9 Ci/mmol). Following incubation, slices were carefully rinsed, dissolved and their radioactive content determined. The assay conditions, established in pilot experiments, assured the conduct of the study in the linear range for both tissue amount and incubation time. ^{3}H-KYN was taken up into slices at a ratio of approximately 5:1 (tissue: medium). Incubation in no Na^+-buffer yielded 32% of the uptake in regular buffer whereas only <3% of the total uptake was detected after incubations at 4°C. Lineweaver-Burk analysis revealed a single component of Na^+-dependent KYN uptake with a K_m of 989 μM and a V_{max} value of 4.6 nmol/h/well (i.e. approximately 3 mg tissue). Specificity of the uptake process was demonstrated by the inability of glutamate, aspartate and several metabolites of the kynurenine pathway (at 1 mM) to influence KYN accumulation. Of the brain regions studied, KYN uptake was found to be the highest in the pyriform and frontal cortex, less prominent in striatum and hippocampus and lowest in the cerebellum.

Preliminary studies in astroglial cultures and striatal P_2 (synaptosomal) fractions showed the existence of a non-Na^+-dependent but temperature-sensitive rapid KYN uptake process in these preparations. The functional significance of the apparently multiple transport systems remains to be explored.

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464.18 INCORPORATION OF KYNURENINE INTO QUINOLINIC ACID: STUDIES IN VIVO AND IN VITRO. A. Eliasson[1], C. Speciale[2] and R. Schwarcz[2] (SPON: E.D. French). [1]Dept. Pharmacology, Karolinska Institute, Stockholm (Sweden) and [2]Maryland Psychiatric Research Center, Baltimore, MD 21228.

Quinolinic acid (QUIN), a regular brain metabolite with possible links to the pathogenesis of human neurodegenerative disorders (Life Sci. 35, 19, 1984), is biosynthesized from tryptophan and kynurenine (KYN) via the kynurenine pathway. KYN but not QUIN can penetrate the blood-brain barrier under physiological conditions. In a first attempt to examine the dynamics of the metabolic relationship between KYN and QUIN, the former was administered i.v. to rats and the effect on serum QUIN concentrations (measured radioenzymatically) monitored continuously in one hour fractions by microdialysis. To this end, an appropriate probe was implanted in the jugular vein of chloral hydrate anesthetized rats and samples were collected for two hours before and at least four hours after KYN administration. In a dose-dependent manner, KYN caused a rapid increase in serum QUIN which appeared to peak during the second hour after KYN infusion. Thus, a baseline serum concentration of 601 ± 53 nM QUIN was elevated to 1.0 ±0.4 μM, 2.4 ±0.6 μM, 6.4 ± 2.0 μM and 80.7 ±26.1 μM QUIN between 60 and 120 minutes following 20, 60, 200 or 600 mg/kg KYN, respectively (N = 4-5 per dose). At the highest dose of KYN, it was possible to examine in more detail both the initial speed of QUIN accumulation and the subsequent clearance of serum QUIN content by assessing five minute fractions. Thus, it was found that half-maximal QUIN concentrations were reached after 30 minutes and that QUIN disappeared from serum with a half-life of 80 minutes.

Parallel in vitro studies were performed in 1 mm thick liver slices (approximately 30 mg). Slices were incubated for 2 hours at 37°C in Krebs buffer, pH 7.4, in the presence of 200 μM or 1 mM KYN, and QUIN production assessed in the medium. At both concentrations, KYN substantially increased extracellular QUIN content (from a control value of 0.6 ±0.1 μM to 2.6 ± 0.1 μM).

These data demonstrate that bioprecursor administration can substantially affect QUIN production and release in the periphery. Similar events can be expected to take place in brain tissue. Implications of this finding for the precipitation of QUIN-induced neurodegenerative events remain to be explored.

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464.19 ON THE DISPOSITION OF INTRAHIPPOCAMPALLY INJECTED ^{3}H-KYNURENIC ACID IN THE RAT. W.A. Turski and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Kynurenic acid (KYNA), a metabolite of tryptophan, is a broad spectrum excitatory amino acid receptor antagonist in the CNS. Since the presence of KYNA in human brain tissue has been tentatively established (cf. Nakamura et al., this meeting), we now investigated the disposition of extracellular KYNA in the brain.

Male rats (200 g) were chronically implanted with unilateral guide cannulae directed towards the dorsal hippocampus. ^{3}H-KYNA (2 μCi; 0.5 μl over 2 min) was injected into the hippocampus of unanesthetized animals 7 days after surgery. Rats were decapitated 5, 15, 30 or 120 min after the end of the injection period. The brains were divided into right and left hemispheres and the following regions rapidly dissected: hippocampus (dorsal, ventral), frontal cortex, thalamus, hypothalamus and striatum. From the same animals, samples of blood, urine, hepatic and renal tissue were obtained. Each tissue sample was homogenized (1:5, w/v) in 6% $HClO_4$, centrifuged and 0.1 ml of the supernatant counted. The pellet was resuspended, washed four times with 6% $HClO_4$ and then dissolved in 1N KOH and counted. No significant amount of radioactivity was detected in the dissolved pellet. Radioactivity was found in all soluble fractions studied. The selected data shown here are expressed as cpm/10mg tissue (N = 5-7 per timepoint).

	5 min	15 min	30 min	120 min
Injected hippocampus	75990	30760	16421	781
Liver	47	28	17	2
Kidney	739	331	134	7
Serum (cpm/10 μl)	209	115	61	4
Urine (cpm/10 μl)	n.d.	n.d.	16863	6460

The identity of the recorded radioactivity was examined in the injected hippocampus and in urine 30 and 120 min after ^{3}H-KYNA injection by TLC and HPLC methodology. In all systems tested, virtually all radioactivity was indistinguishable from authentic KYNA.

In separate in vitro experiments, the existence of uptake processes for KYNA was investigated. Hippocampal slices (500 μm thick) were incubated for 2 hours in 1 ml Krebs-Ringer buffer, pH 7.4, at 37°C or 4°C with 100 nM or 100 μM ^{3}H-KYNA (0.2 μCi) in the presence or absence of Na^+. No distinct accumulation of ^{3}H-KYNA occurred under any of these experimental conditions.

Our data demonstrate that extracellular KYNA can be rapidly removed from the rat brain by processes other than uptake or metabolism.

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464.20 **INVESTIGATIONS INTO THE CONFORMATION OF ACETYLCHOLINESTERASE.** S. N. Abramson[1], Z. Radić*[1], H. A. Berman*[3], T. S. Vedvick*[2], and P. Taylor[1]. University of California-San Diego, Departments of Pharmacology[1] and Chemistry[2], La Jolla, CA 92093; State University of New York-Buffalo[3], Department of Pharmacology, Buffalo, NY 14260.

The asymmetric form of acetylcholinesterase (AChE) from *Torpedo Californica* is composed of disulfide-linked tetramers of 68,000-Dalton subunits that are linked to a collagen-like tail by disulfide bonds. It is possible to release the tetramers from the collagen-like tail with mild proteolysis, resulting in an 11 S sedimenting species. The complete amino acid sequence of the subunits of 11 S AChE has been determined by sequencing the protein and a cDNA clone. Each monomer contains 8 cysteines. Sequencing of tryptic peptides from reduced and nonreduced 11 S AChE has revealed that there is one free cysteine, three intramolecular disulfide bonds, and one intermolecular disulfide bond. However, the cysteine(s) involved in formation of the 11 S tetramer and in attachment of the tetramer to the collagen-like tail have not yet been identified. Although a complete understanding of the conformation of AChE will require X-ray crystallography, important constraints on the conformation of AChE can be determined by investigating the disulfide bonds of the native enzyme, and also through the use of site-directed cross-linking agents. We have undertaken such studies to determine which cysteine(s) are involved in formation of the 11 S tetramer and in attachment of the tetramer to the collagen-like tail, and to determine which amino acids are in spatial proximity to the active site serine.

We have observed that digestion of nonreduced 11 S AChE with cyanogen bromide results in formation of a 30,000 Dalton fragment that is composed of two disulfide-linked 15,000 Dalton peptides. This disulfide-linked cyanogen bromide fragment is not predicted from the known sequence of AChE or from the known disulfide bonds. It therefore contains additional intramolecular and/or intermolecular disulfide bonds. The 30,000 Dalton fragment was purified by preparative SDS-polyacrylamide gel electrophoresis, isolated by electroelution, and digested with trypsin. The tryptic peptides were then separated by reverse-phase HPLC and sequenced. Their position within AChE has enabled us to identify Cys^{231} as the most likely cysteine involved in formation of the 11 S tetramers and in attachment of the tetramers to the collagen-like tail.

We have used a photoactivatable organophosphate inhibitor (NAP) of AChE to determine which amino acid residues of 11 S AChE are near the active site serine. The active site serine was phosphonylated with NAP in the dark. Prior to photolysis the enzyme activity could be reactivated with pralidoxime. After photolysis, however, the enzyme activity could not be regenerated, suggesting that NAP has been covalently photo-incorporated into the active site. Enzyme labeled with NAP and then photolysed was digested with trypsin, and the tryptic peptides purified by reverse-phase HPLC at pH 7 and pH 2. NAP-labeled peptides were identified by their absorbance between 400nm and 500nm, and subjected to sequence analysis. Labeled fractions contained the active-site peptide and an additional peptide, presumably from the spatial proximity of the active site.

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464.21 TISSUE DISTRIBUTION OF [^{3}H]HI-6 IN THE PRESENCE OF ATROPINE IN ORGANOPHOSPHORUS POISONED MICE. G. Amitai, L. Raveh*, I. Barness*, A. Balan*, S. Chapman*, T. Kadar*, G. Cohen* and S. Shapira*. Dept. of Pharmacology, IIBR, Ness Ziona 70450, Israel.

HI-6 [4-carbamoyl 2'-aldoxime 1,1'-oxydimethylene di(pyridinium chloride) is a powerful reactivator of AChE inhibited by various organophosphates (OP). Efficient antidotal treatment of severe OP poisoning in laboratory animals is obtained by the use of HI-6 *in combination* with atropine. Since atropine may affect certain hemodynamic parameters it was of interest to study the in-vivo distribution of HI-6 in the presence of atropine. We have, therefore, compared the time-course for tissue distribution of [^{3}H]HI-6 either in the presence or absence of atropine in non-poisoned and OP-poisoned mice. [^{3}H]HI-6 (19.5Ci/mmole) was prepared from tritium-labelled 2-pyridine aldoxime obtained from reduction of 6-bromo 2-(formyl diethylketal) pyridine. The authentic [^{3}H]HI-6 solution was diluted with unlabelled HI-6 to yield 85mCi/mmole and injected i.m. (28µmole/kg) to albino mice with or without atropine (22µmole/kg), 5 min after s.c. administration of either saline or soman (1xLD50). Blood samples were withdrawn from animals (n=3) sacrificed at 1, 5, 15, 30, 60, 120 min and 24h post HI-6 administration. Brain, lungs, kidneys and liver were immediately dissected out, solubilized (100mg/ml) and 100µl aliqouts were counted. Quantitative whole-body autoradiography (QWBA) was performed with 40µm frozen sections by using a computerized densitometer. In blood, lungs, kidneys and liver, peak levels of [^{3}H]HI-6 were obtained within 5 minutes and in brain within 15 minutes. The highest level (100.8±4.8 nmole/g tissue) of [^{3}H]HI-6 was obtained in kidneys, while the lowest level (2.2±0.5 nmole/g tissue, uncorrected for blood content) was found in brain. In the presence of atropine, the clearance of [^{3}H]HI-6 from blood was slowed down. This effect was more pronounced in soman poisoned animals. Accordingly, peak levels of [^{3}H]HI-6 in kidneys and liver of poisoned animals were obtained only at 15 min as compared to 5 min after administration of HI-6 alone to non-poisoned animals. QWBA of animals administered with [^{3}H]HI-6 alone, displayed labelling within 5 min in the following order: kidney > lungs > liver. After 2h, radioactive density in these organs diminished, while considerable labelling appeared in the intestinal lumen. In contrast, the densitometric analysis of soman poisoned animals displayed significantly lower radioactivity within 5 min in the kidney, lungs and liver. In these animals, residual labelling was detected after 2h in the renal medulla and in the urinary bladder whereas radioactivity in the intestinal lumen was less pronounced. Our data suggest, that atropine in soman-poisoned mice reduces the rate of metabolism and clearance of HI-6. (Supported in part by DAMD17-84-C-4016)

464.22 SEQUENTIAL METABOLISM OF ANGIOTENSINS II AND III BY MEMBRANE PEPTIDASES FROM RAT BRAIN. J.M. Hanesworth*, J.W. Harding, and R.H. Abhold. Dept. of Vet. Comp. Anat. Pharmacol. Physiol., Washington State University, Pullman, WA 99164-6520.

This study examined the metabolism of [^{125}I]angiotensin II ([^{125}I]AII) and [^{125}I]angiotensin III ([^{125}I]AIII) by homogenates of rat brain. Using HPLC methodologies, membrane-bound angiotensinase activities were monitored. In the metabolism of [^{125}I]AIII, sequential production of [^{125}I](3-8)AII, [^{125}I](4-8)AII, and [^{125}I]tyrosine ([^{125}I]Tyr) was observed within the first 5 minutes. On longer incubation, a [^{125}I](2-7)AII fragment was also produced. The first step in the metabolism of [^{125}I]AIII was markedly inhibited by AII, AIII, and (3-8)AII. In contrast, [^{125}I]AIII, [^{125}I](2-7)AII, [^{125}I](2-4)AII and [^{125}I]Tyr were the fragments sequentially produced in the metabolism of [^{125}I]AII. The formation of [^{125}I]AIII appears to be the rate limiting step in this sequence. AII, AIII, (3-8)AII, (4-8)AII, and (5-8)AII were relatively effective in preventing the formation of [^{125}I]AIII. The subsequent conversion of [^{125}I]AIII to [^{125}I](2-7)AII could only be inhibited with AII and AIII. These data suggest that exogenously applied [^{125}I]AIII was preferentially metabolized by one or more aminopeptidases whereas the [^{125}I]AIII generated from [^{125}I]AII was preferentially metabolized by one or more carboxypeptidases. Though the metabolism of generated [^{125}I]AIII is clearly different than that of applied [^{125}I]AIII, it is likely that the two pathways reflect differences in substrate concentration and enzyme affinity. The specificity of the enzymes governing the initial steps in each pathway is evidenced by the differential capability of AII, AIII, and several angiotensin peptide fragments to selectively inhibit product formation. These data demonstrate that [^{125}I]AII and [^{125}I]AIII are metabolized by membrane-bound angiotensinases in an orderly and sequential manner. The metabolic pathways described are consistent with the notion that specific angiotensinase activity may modulate the physiological effects of angiotensin peptides.

464.23 NEUROCHEMICAL CORRELATES OF TRIGEMINAL, POST-TRAUMATIC, AND CHRONIC MIGRAINOUS NEURALGIA. W.H. Sweet[1], A.J. Bouckoms[2] and W.R. Matson[3]. Neurosurgical[1] and Psychiatry[2] Services, Mass. Gen. Hosp., Boston, MA 02114, USA and [3]ESA, Inc., Bedford, MA

Aim: Using a dramaticly advanced method for assaying picogram to femtogram amounts of literally hundreds of chemicals in biological samples of less than 1 ml we are seeking to define specific neurochemical profiles in the cerebrospinal fluid (CSF) of the trigeminal cistern in various types of facial pain. This tiny cistern of ~0.6 ml is a blind csf-filled sleeve extending forward from the posterior fossa around the trigeminal rootlets to the posterior edge of the trigeminal ganglion. Its stagnant fluid has an elevated total protein and may contain compounds specificly related to processes in rootlets and ganglion.

Method: Most of our data are from patients with either trigeminal, post-traumatic or chronic migrainous neuralgia. We are analyzing samples from over 200 patients, comparing the chemical content of trigeminal cistern csf with much larger samples, sequentially obtained via the same needle, of fluid passing forward from the large cisterns in the posterior fossa. An array of 16 coulometric electrode sensors permits up to 16 coeluting compounds to be determined simultaneously. In practice 5-12 can be resolved in our current liquid chromatograph.

Results: To date 326 compounds have been identified and measured quantitatively. Measuring 31 different metabolites of tryptophane and tyrosine permits determination of flux rates in these systems. Similar analyses are in progress for the monoamines, epinephrine, norepinephrine, dopamine and serotonin. To date the neuropeptides met- and leu-enkephalin, somatostatin-14 and cholecystokinin-8 have been measured. Tentatively we have identified distinctive chemical insignia involving 16 compounds characteristic of each of the 3 disorders mentioned. Hypotheses anent mechanisms of pain in each disorder may evolve as we further categorize the data evolving.

464.24 CALCIUM METABOLIC FUNCTION IN PLATELETS OF DEPRESSED AND CONTROL SUBJECTS. C.L. Bowden, M.A. Javors, and E. Seleshi. Department of Psychiatry, University of Texas Health Science Center. San Antonio, Texas 78284.

Platelets have been used as a peripheral model for central nervous system nerve endings because they take up, store, and release serotonin, a neurotransmitter found in brain. The release of neurotransmitter in brain cells and platelets is regulated by intracellular free calcium concentration ([Ca^{++}i]). The purpose of this study was to compare calcium metabolic function in platelets from depressed and control subjects. Resting [Ca^{++}i], as well as the maximal change in [Ca^{++}i] in response to 10 µm epinephrine, 50 nM PAF (platelet activating factor), and 1 unit/ml thrombin, were measured at [Ca^{++}ext]'s of zero (50 µM EGTA added), approximately 1 µM, and 1 mM using the Ca^{++}-chelating fluorescent dye Fura-2. Resting levels of [Ca^{++}i] ranged from 65 to 320 nM in subject groups when [Ca^{++}ext] was 1 µM. When [Ca^{++}ext] was zero, resting [Ca^{++}i] ranged from 16 to 163 nM, with a tendency of depressed and manic subjects to have lower values than control subjects. Thrombin (1 u/ml) caused a smaller increase in [Ca^{++}i] in bipolar depressed and manic subjects than in control subjects. Based on differential change in [Ca^{++}i] in experiments conducted with [Ca^{++}ext] of 1 mM and zero, we determined that approximately 90% of the rise in [Ca^{++}i] comes from the influx of extracellular Ca^{++}. Following four weeks treatment with lithium, both resting and thrombin stimulated [Ca^{++}i] levels were reduced in four of five bipolar subjects (pretreatment resting [Ca^{++}i] was 143 ± 72 nM; treatment resting [Ca^{++}i] was 80 ± 33 nM). The findings indicate that differences linked to depressive disorder diagnosis and treatment response may be identifiable in measurement of dynamic changes of [Ca^{++}i] in platelets. This system appears to be suitable for study of receptors linked to PI hydrolysis, rise in [Ca^{++}i], and the dynamic processes of Ca^{++} extrusion and reuptake into the dense tubular system of platelets. (Funded by a grant from the Meadows Foundation, Dallas, Texas).

465.1 EFFECTS OF EXPERIMENTAL TRAUMATIC BRAIN INJURY ON PROSTAGLANDIN LEVELS IN RATS. D.S. DeWitt*, D.L. Kong*, B.G. Lyeth*, R.L. Hayes, L.W. Jenkins*, D.S. Prough* (SPON: J. Butterworth). Dept. of Anesthesia, Bowman Gray Sch. of Med., Winston-Salem, NC 27103; Div. of Neurosurgery, Med. Coll. of VA, Richmond VA 23298.

Increases in brain prostaglandin (PG) levels may contribute to some of the functional and morphological abnormalities that are observed following fluid percussion injury in cats (Ellis, et al., J Neurochem, 37:892-896, 1981). In order to determine whether similar increases in prostaglandins occur in a rodent model of fluid percussion injury, rats were anesthetized with pentobarbital and prepared for injury (Dixon, et al., J Neurosurg, in press). Twenty-four hours later, rats were lightly anesthetized with methoxyflurane, injured (2.3 atms.) and sacrificed 5 (n=6) or 15 (n=7) minutes after injury. Control rats (n=6) were prepared for injury but not injured. Thirty seconds prior to sacrifice by decapitation into liquid nitrogen, all rats were injected with indomethacin (3 mg/kg, i.v.) to prevent post-mortem PG synthesis. Brains were removed, weighed and homogenized in phosphate buffer with indomethacin (50 ug/ml). PGE_2 and thromboxane (TxB_2) levels were determined using double-antibody radioimmunoassay (McCosh, et al., Prostaglandins, 12:471-486, 1976).

Post-traumatic hind limb convulsions were observed in 4 of the 7 rats during the 15 minutes before sacrifice. These animals were analyzed separately.

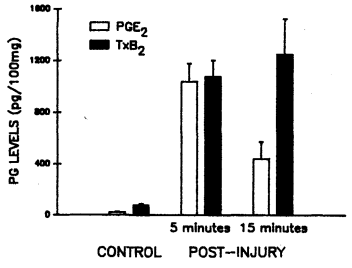


Figure. Effects of fluid percussion brain injury on hemispheric PGE_2 and thromboxnae (TxB_2) in rats. Values are mean ± SEM.

These transient increases in PG levels are consistent with a recent report of elevated PG synthesis in a different rodent model of traumatic brain injury (Shohami, et al., J Cereb Blood Flow & Metab, 7:58-63, 1987). Supported by NIH grant NS19355.

465.2 PROXIMAL NEUROFILAMENTOUS SWELLINGS AND MITROCHONDRIAL ALTERATIONS FOLLOWING HIGH DOSE CARBON DISULFIDE ADMINISTRATION. B.G. Gold[1], J.W. Griffin[2], S.B. Andrews[3] and C. Zola*[1], [1]Rutgers University College of Pharmacy, Piscataway, NJ 08854, [2]The Johns Hopkins University School of Medicine, Baltimore, MD 21239 and [3]NINCDS, NIH, Bethesda, MD 20892.

Distal neurofilamentous axonal swellings are observed in a variety of human and experimental conditions, including those produced by repeated exposures to carbon disulfide (CD), acrylamide (AC) and 2,5-hexanedione (HD). We have previously demonstrated that high dose injections of AC (Gold, Griffin and Price, J. Neurosci., 1985) and HD (Gold and Griffin, J. Neuropath. Exp. Neurol., 1986) produce proximal as opposed to the distal neurofilamentous swellings observed following repeated administrations. Thus, it was important to test whether high dose exposures to CD can similarly produce proximal swellings.

Initial studies were performed on ten 6-week old rats given CD (1gm/kg, i.p.) in olive oil for 1-3 days and perfused with 5% glutaraldehyde either 24 hours or 7 days following the last injection. The entire sciatic nerve from the lumbar spinal cord to the muscles of the hindfeet was sampled and prepared for light and electron microscopy. Appropriate sections were prepared for X-ray microprobe analysis. In latter studies, three 3-week old rats were given a single injection of CD, as above, in 20% dimethylsulfoxide (DMSO) and perfused at 7 days.

High dose administrations of CD produced neurofilamentous axonal swellings. In preliminary studies, these were observed in the ventral white matter of the lumbar spinal cord and in the proximal portion of peripheral nerves, although only some (<20%) axons in the spinal roots were affected. Electron microscopy revealed that many axons contained enlarged mitochondria with dense particles located between the inner and outer membranes; mitochondria in associated Schwann cells appeared normal. X-ray microprobe analysis revealed that these particles were composed mostly of zinc, and barium; copper could not be accurately measured in these preparations. CD, in 20% DMSO, produced modest swellings in the proximal regions of almost all sensory and motor fibers. Quantitation of neurofilament numbers in these regions revealed a significant ($p<.05$) increase (approx. 50%) in density compared with age-matched controls.

Proximal neurofilamentous axonal swellings are produced following high dose CD injections. The significance of the metal deposits remains unclear. However, it is interesting to note that their location in mitochondria coincides with the copper/zinc isoenzyme of superoxide dismutase and that inhibition of copper/zinc-dependent enzymes have been implicated in the pathogenesis of CD neurotoxicity. Supported by NIH grant ES 04078.

465.3 IMMUNOELECTRON MICROSCOPIC INVESTIGATION OF COLD INJURY IN THE GERBIL BRAIN. M. Maeda*, F. Akai*, S. Nishida* and T. Yanagihara. Dept. of Neurology, Mayo Clinic, Rochester, MN 55905 and Dept. of Pathology, Kinki University, School of Medicine, Osaka, Japan

In cold injury, an extensive vasogenic cerebral edema with extravasation of albumin occurs. In the present investigation, we studied the distribution, particularly intracellular localization, of albumin by electron microscopic immunocytochemistry and correlated the finding with morphologic alterations. A cold injury was produced in the parietal cortex of the gerbil. The immunocytochemical procedure was carried out with a brain section prepared by a vibratome by using an antiserum for gerbil albumin and the peroxidase-antiperoxidase complex. Ultrastructural investigation was focused in the area surrounding the cold injury.

Thirty minutes after cold injury, extravasated albumin was already observed in the extracellular space and within a few neuronal cell bodies and dendrites. There were mild morphological alterations in the neuronal cell bodies with mild dilatation of endoplasmic reticulum (ER) and swelling of mitochondria. In some dendrites, the distribution of microtubules was irregular and the number was reduced. The positive reaction for albumin in the neuronal cell bodies was dispersed throughout the cytoplasm but corresponded most closely to the distribution of polyribosomes. No reaction was observed inside the nuclei, mitochondria or the cistern of the Golgi complex. In the dendrites, the distribution of albumin corresponded most closely to the distribution of microtubules. After 1 day, the neuronal cell bodies with albumin deposition showed marked dilatation of ER with elongation and invagination into the nuclear matrix, swelling of mitochondria, disaggregation of polyribosomes and reduction of cytoplasmic electron density. Deposition of albumin was observed in the dendrites which had relatively normal or irregular alignment of microtubules as judged by the pattern of albumin deposition. Severely degenerated dendrites without albumin deposition were also observed. After 3 days, slight improvement was observed in the structure of the nerve cell bodies with albumin deposition. Albumin in the extracellular space was still present. After 7 days, the number of the nerve cell bodies and dendrites with albumin deposition was reduced. Some neurons with albumin deposition showed clear evidence of recovery with reduction of the size of ER and mitochondria, and restoration of polyribosomes and cytoplasmic electron density. Albumin was not detected in the extracellular space any longer.

The present investigation demonstrated the morphological alteration of the neuronal structure associated with influx of albumin and probably of water and electrolytes.

465.4 **IN VITRO DIFFERENCES IN CELLULAR PROLIFERATION IN AREAS PROXIMAL OR DISTAL TO INJURY IN THE ADULT RAT BRAIN.** *I.R. Katz*, L. Iacovitti and D.J. Reis,* (SPON: J. Chan). Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Cellular proliferation has long been recognized as a consequence of brain injury. However, due to limitations of *in vivo* methods, the course of events and identification of responding cells have been uncertain. In this study we sought to determine 1) the extent of the proliferative response (PR) *in vitro* by measuring DNA incorporation 2) the proportion of responding phenotypes and 3) whether the PR is limited to the immediate area of injury or whether it can be detected in distant regions, either those connected to the lesion by neuronal pathways or those totally unrelated to the lesion site. Ibotenic acid, a neuron-specific excitotoxin was stereotaxically injected into 3 sites (4 µg/0.4 µl) of one caudate nucleus (CN) in anesthetized adult rats. Since ibotenic lesion in CN results in degeneration of the striato-nigral pathway and delayed transneuronal death in the substantia nigra (SN) (Saji and Reis, Science 235:66, 1987), we measured the time course of PR in CN and SN as well as in unrelated brain regions remote from the lesion: hypothalamus (HT) and cortex (CX). Rats were decapitated between 6h and 60d post-lesion. Tissue was dissected, dissociated, plated and incubated in 3-4d cultures. ^{3}H-thymidine incorporation (0.2 µCi, 6.7 Ci/mmol) was measured in 4 replicate cultures on d4. Time course of PR was measured between d1-d28 post-lesion. The PR of cells from unlesioned rats was designated as baseline response measuring 823 DPM. In the ipsilateral CN (CN_I) there was a 5-fold increase (4,450 DPM) in the PR within 6h post-lesion. Interestingly, PR from contralateral CN (CN_C) also increased. Peak PR was reached by d9 post-lesion (CN_I: 21,100 DPM, CN_C: 3,775 DPM). PR did not return to uninjected levels but persisted up to 60d (CN_I:4450 DPM; CN_C:2386 DPM). As compared to CN_I, PR in cells from SN_I lagged in onset (7d vs. 6h) yet peaked around the same time and was significantly higher than PR from SN cells of unlesioned controls (9,488 DPM vs 3,516 DPM). Responses from HT and CX as well as SN_C did not rise above control levels. Immunocytochemical labeling with monoclonal antibodies to specific cell markers demonstrated that cultures of CN_I and CN_C, at peak period post-lesion, contained >75% blood monocytes, macrophages and T-helper lymphocytes (OX-35$^+$ and w3/25$^+$ cells), the rest being GFAP$^+$ astrocytes and Thy 1.1$^+$ fibroblasts. Autoradiographic labeling experiments confirmed that most proliferating cells belonged to the w3/25$^+$ phenotype. In addition, we used a cytolytic monoclonal antibody OX-35 to demonstrate that in the presence of complement, > 50% of cultured cells derived from both CN_I and CN_C were eliminated in a proliferative assay. Immunocytochemical studies on SN_I cells showed that at peak response, about 30% of the proliferating cells were of hematic origin, while 70% consisted of glia. During all other times studied, 90% of PR of SN_I cells were of glial origin. We conclude that 1) the PR to brain injury in CN has a rapid onset, peaks within 2 weeks and persists indefinitely; 2) the majority of proliferating cells that respond to selective neuronal injury in CN are not intrinsic to CNS but are of hematic origin; and 3) in contrast, the PR in distant but related regions (SN) lags in onset, is of short duration and involves mostly glial cells, while no PR is observed in unrelated brain regions.

465.5 CONSTRICTION OF THE SQUID GIANT AXON AFTER TRANSECTION. P. E. Gallant. Laboratory of Neurobiology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health at the Marine Biological Laboratory, Woods Hole, MA 02543.

After transection both ends of a cut axon reform a high resistance seal (Meiri et al, Brain Res. 19:183-198, 1983). The reformation of this high resistance seal requires calcium and the activation of a calcium dependent phospholipase (Yawo H. and Kuno M., J. Neuroscience 5:1626-1632 1985). In addition to changes in the lipid constituents at the cut end, exposure of the cut end to the extracellular ions may also induce changes in the cytoskeletal proteins and the size and shape of the axon which are in part maintained by the cytoskeletal proteins. In the squid giant axon transection led to a softening of the exposed axoplasm and a pinching off (constriction) of the cut end. Constriction occurred when the axon was cut in the presence of external medium (an artificial squid blood), but failed to occur when the axon was cut in the presence of internal medium (composed of the ions normally present inside the axon). This difference in the amounts of constriction was largely due to the different cation composition of the two media. Specifically constriction required the mM concentrations of calcium ion, and the relatively low potassium to sodium ratio present in the external medium. Softening of axoplasm appeared to be necessary for constriction since constriction occurred only under conditions which induced axoplasmic softening. Axoplasmic softening was not sufficient for constriction, however, since constriction could be inhibited (for example by reducing ATP levels with dinitrophenol) without inhibiting axoplasmic softening. These results suggest that exposure of the cytoskeletal proteins to the extracellular environment induces cytoskeletal changes (like axoplasmic softening and perhaps a contraction of some of the cytoskeletal proteins) that contribute to the response and perhaps repair of a damaged axon.

465.6 ENDOTHELIAL CELL CHANGES IN RESPONSE TO EXPERIMENTAL HYPERTENSION OR DIABETES SINGLY OR IN COMBINATION. P.A Grady. Dept. Neurology, Univ. of Maryland Sch. of Med., Baltimore, Maryland 21201

This study tests the effects of hypertension and diabetes singly and in combination on the cerebral blood vessels. Adult male spontaneously hypertensive rats (SHRs) were made diabetic by injection of streptozotocin (45 mg/kg). Adult male Wistar-Kyoto rats were used as controls and also rendered diabetic. Animals were maintained for up to three months with either or both of these factors. Blood pressures were monitored weekly on the caudal artery using the indirect tail-cuff method. Blood glucose was monitored weekly using the o-toluidine method.

After three months in experimental groups, animals were anesthetized and perfused through the left ventricle of the heart with fixative. Major cerebral arteries were removed and prepared for scanning electron microscopy (SEM). Comparison of vessel changes in response to these experimental risk factors was made among the hypertensive diabetic rats, hypertensive nondiabetic rats and normotensive diabetic rats in age-matched groups for the same duration of risk exposure.

After three months of exposure to both factors in combination, the major cerebral arteries showed distinct signs of alteration of luminal surfaces. Endothelial surfaces were swollen or frothy in appearance with areas of frank endothelial injury superimposed on the irregular surfaces. Systemic vessels were affected more severely, but the pattern of abnormalities was essentially the same. Examination of age-matched animals with the single risk factor of hypertension for the same duration revealed frank endothelial injury and subtle restructuring of endothelial surfaces, but luminal surfaces did not appear swollen as in the diabetic hypertensives. The vessels of age-matched animals with diabetes alone for the same period of time showed a distinctly cobbled or swollen appearance, but the absence of frank endothelial injury. These changes were consistent.

In conclusion, these findings provide evidence which strongly supports the idea that each of these important risk factors for the development of cerebral vascular disease and stroke results in a different type of injury or structural change in the early periods which we have studied, and that the combination of these two risk factors, hypertension and diabetes, results in lesions that are different in nature from those seen in vessels exposed to each risk factor singly. These structural changes provide the basis for altered hemodynamic patterns and increased thrombogenic potential. These findings are important in our further understanding of the mechanism of the early vascular changes resulting from these two major risk factors for developing a stroke. Supported in part by NIH grant NS 16332.

465.7 LEVELS OF Na AND OTHER ELEMENTS IN SUBCELLULAR COMPARTMENTS OF SCIATIC NERVE FROM NORMAL AND DIABETIC RATS. J. Lowery*, R.M. LoPachin, A.J. Sauberman* and J. Eichberg, University of Houston, Houston, TX 77004 and University of Texas Health Science Center, Houston, TX 77025.

Experimental diabetic neuropathy is characterized by reduced peripheral nerve conduction velocity which is manifested as early as 2 weeks after induction of diabetes, diminished Na^+K^+-ATPase activity and structural abnormalities of the axo-glial junction (Sima et al (1986) J. Clin. Invest. 77:474). Electrophysiological data suggest that in experimental diabetes both the axoplasmic Na^+ concentration and K^+ permeability are increased (Brismar and Sima, (1981) Acta Physiol Scand. 113:499). In order to obtain more direct evidence for these changes, we have used x-ray microprobe analysis to determine the concentrations of elemental Na,K,P,Cl and Ca in subcellular compartments of sciatic nerve from normal and streptozotocin diabetic rats. Axonal cytoplasm, mitochondria, myelin and extra-axonal space were examined following preparation of frozen, ultrathin, unfixed transverse and longitudinal sections. Upon analysis of transverse sections, normal axoplasm exhibited (in μmoles/g dry weight) low Na (96 ± 10) and high K (2012 ± 65) levels. The contents of these elements in extra-axonal space were 1697 ± 179 and 375 ± 119. In axoplasm from nerves of rats that had been diabetic for 10 weeks, Na showed no significant change (117 ± 14) but was substantially elevated (202 ± 32) after 20 weeks. K levels were not significantly altered, but Ca tended to increase. Mitochondria from diabetic rat nerve displayed elevated Na and Ca and decreased K, whereas the elemental content of myelin was not consistently different. The alterations in Na levels in diabetic nerve were much less pronounced than those observed either in ischemic nerve or following axotomy. Limited examination of longitudinal sections suggested that Na levels are highest at the node of Ranvier and decrease progressively with increasing distance from the node. These data support the conclusion that intra-axonal Na accumulation occurs in chronic experimental diabetes at a time well after the reported onset of decreased conduction velocity. The possibility exists that the increased Na levels are associated with disappearance of normal axo-glial junctions. (Supported by NIH grants DK30577 and ESO3830).

465.8 IN VIVO EFFECTS OF ESTROGEN, PROGESTERONE AND THE ANTIPROGESTERONE RU38486 ON MENINGIOMAS IN NUDE MICE. M. M. Poor, Jr.*, D. W. Beck. Division of Neurosurgery, University of Iowa Hospitals, Iowa City, Iowa 52242.

We have used in vivo subcutaneously implanted meningiomas in nude mice to evaluate growth of these tumors during hormonal manipulation. Human meningioma tissue was obtained from two patients. Portions of the tumor were used for estrogen and progesterone binding protein determinations by Scatchard analysis. The remaining portions of each tumor were divided into equal fragments of 3x3x3 mm. These were implanted over the left scapula in a subcutaneous pocket in 20 nude mice weighing 35-45 mg following intraperineal chloral hydrate anesthesia. The 20 mice used for each experiment were divided into 4 groups of 5 mice. These groups received oral estrogen or progesterone in doses of 7.5 mg/kgm/day or the antiprogesterone RU38486 at a dose of 10 mg/kgm per day. Five mice served as controls. The meningoma volumes were defined by a formula $V=W^2xL/2$ where V represented the total volume, W the width and L the length by Anthropometric measurements. These tumors were measured 2 weeks following implantation, then on a monthly basis for 3 months. Tumor volumes were compared at the beginning and end of the experiment and a growth index (GI) was calculated as GI=Vf÷Vi with Vf representing the volume determination at the end of the experiment and Vi representing the volume of the tumor 2 weeks following implantation. Results were compared by student "t" tests for unpaired data.

The initial tumor tested was found to have progesterone binding proteins in concentrations of 30.6 fM/mg cytosol protein and no estrogen binding proteins. Growth indices of 1.25 in control animals and 1.49 in progesterone treated animals were significantly greater than the growth index of 0.89 in the RU38486 treated animals. Animals treated with beta estradiol showed no significant changes in growth indexes from any group. The second tumor was also found to have progesterone binding proteins at a concentration of 48.8 fM/mq protein. However, in this group, growth of the meningiomas was not seen and growth indexes were less than 1 for all groups. There was no statistically significant difference in the growth indexes of all these groups compared to one another. These results suggest that in certain meningiomas, hormonal manipulation may be of value in controlling tumor growth. At this time there is no known method of determining which meningioma will respond to hormonal manipulation. Further in vitro and in vivo trials may confirm the efficacy of antiprogestational compounds in the treatment of inoperable or recurrent meningiomas.

465.9 EVALUATION OF TUMOR NECROSIS FACTOR: ANTI-VIRAL ACTIVITY AND EFFECT ON LATENT HSV-1 GANGLIONIC INFECTION IN RABBIT EYES. G. Gallez-Hawkins*, M. Narachi* and M.D. Trousdale* (SPON: E.F. Hawkins). Doheny Eye Foundation, USC School of Medicine, L.A., CA 90033 and Amgen Corp., Thousand Oaks, CA 91320.

Tumor necrosis factor (TNF) is known to have anti-tumor and anti-viral activity (Mestan et al., Nature 323:816, 1986). The therapeutic effect of topical human recombinant TNF treatment was evaluated in New Zealand white rabbits with confirmed latent HSV-1 infection. Beginning 7-12 months after confirmed ocular infection, TNF was administered topically (1×10^5 U/eye/day) alone and in combination with human recombinant interferon gamma ($IFN\text{-}\gamma_{4A}$) (1×10^6 U/eye/day), during which time the rabbits underwent concurrent stimulation by bilateral iontophoresis of 6-hydroxydopamine plus topical epinephrine on the cornea to intentionally reactivate latent virus.

Following placebo (saline) treatment, 9 of 10 eyes shed virus in tears during one week of concurrent reactivation and therapy. Similar results were obtained with animals receiving TNF or $TNF/IFN\text{-}\gamma_{4A}$ (9/10 and 11/12 eyes were HSV positive for TNF and $TNF/IFN\text{-}\gamma_{4A}$, respectively).

The severity of ocular disease was generally greater after intentional reactivation. Topical treatment with TNF or $TNF/IFN\text{-}\gamma_{4A}$ did not suppress the development of ocular disease.

No significant difference in incidence of HSV-positive trigeminal ganglia was detected following concurrent reactivation attempts in the placebo, TNF or $TNF/IFN\text{-}\gamma_{4A}$ treated groups (i.e., 55%, 40% or 46%, respectively, were virus positive). However, the time required for appearance of typical HSV cytopathic effects (CPE) in cocultivated trigeminal ganglia cultures was delayed by treatment (i.e., CPE was detected at 5, 10 and 12 days postincubation for placebo, TNF and $TNF/IFN\text{-}\gamma_{4A}$ treated groups).

In summary, topical TNF treatment did not (1) significantly alter the frequency of HSV shed into tears following reactivation, or (2) suppress the development of ocular disease. However, the time required for HSV detection in cultured trigeminal ganglia was delayed.

465.10 AN in vivo 31P NMR STUDY OF THE ACCUMULATION OF PHOSPHOETHANOLAMINE BY NEUROBLASTOMA CELLS IN A/J MICE AS A FUNCTION OF THEIR LOCATION IN THE TUMOR P. Glynn* and S. Ogawa* (SPON: R. L. Chappell)AT&T Bell Laboratories, 600 Mountain Avenue, Murray Hill, NJ 07974. We have reported large changes in the concentrations of phosphoethanolamine (PEt) and phosphocholine (PCh) in mouse neuroblastoma cells, the N1E-115 clone of the C1300 tumor, in tissue culture when they were induced to differentiate with DMSO (Glynn, P., et al, Soc, Neurosci. Abstr., Vol. 12, Part 1, p.320, 1986). In these non-dividing differentiated cells the PEt level exceeded the cytosolc ATP level by more than a factor of 2. A similar high level of PEt was reported in human neuroblastoma in vivo (Maris, J.M., et al, Soc. Magnetic Resonance in Medicine, 3rd Annual Meeting, P.501, 1984). Tumors in general are known to be heterogeneous morphologically and metabolically. Therefore it is possible that the high PEt concentration in tumor originates from the differentiated cells and not from the dividing ones and that these cell types are physically segregated. Using 31P NMR, we investigated the metabolic character of the tumors induced in the neck of A/J mice by subcutaneous injection of N1E-115 cells. The tumors became visually noticeable several weeks after the implantation. When the tumors were examined visually, one could see a highly vasculated cortex with the consistency of liver and slurry of neuroblastoma and red blood cells at the core. Neuroblastoma cells in the slurry were spherical in shape, When they were transferred to standard tissue culture conditions, however, they did not attach to the tissue culture plates in contrast to the N1E-115 cells which do attach. The addition of DMSO did not induce differentiation either. This suggested that there were alterations in the tumor cell line. The in vivo 31P NMR spectra of the cortex of these tumors showed a large phosphomonoester and Pi peaks, but essentially no creatine phosphate. The Pi peak indicated the cellular pH was 7.2 to 7.0. The major component of the phosphomonoester peak was PEt as determined in the chloroform-methanol extracts of the tumors. The spectra of a whole tumor varied to some extent with the age or the size of the tumor and with the metabolic conditions of the animal. Variation appeared in the amounts of phosphomonoester, Pi and creatine phosphate and also in the pH values. We have not yet been able to obtain the spectra of the slurry because of the spacial complexity and the small volume fraction of the neuroblastoma cells in the slurry relative to the cortex. We are interested in finding out which anatomical site of a tumor (the cortex of the slurry) contains the dividing cells for the growth of the tumor.

465.11 FUNCTIONAL SIGNIFICANCE FOR THE SEQUENCE HOMOLOGY BETWEEN HUMAN IMMUNODEFICIENCY VIRUS AND NEUROLEUKIN. M.R. Lee,* D.D. Ho* and M.E. Gurney. Depts. of Neurology and Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637 and Dept. of Medicine, Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, CA 90048

Neurotrophic factors are important for the development and maintainance of the nervous system. Nerve growth factor (NGF) remains the best characterized neuronal growth factor. However, numerous tissue culture experiments have demonstrated that *in vitro* survival of embryonic neurons can be supported by factors other than NGF. We have recently sequenced and cloned a 56,000 dalton growth factor named neuroleukin (NLK). NLK is both a neurotrophic factor and a lymphokine product of T cells.

We find that NLK exhibits partial amino acid sequence homology to a highly conserved region of the external envelope protein of the human immunodeficiency virus (HIV), the etiological agent of the acquired immunodeficiency syndrome (AIDS). Neurological dysfunction, termed AIDS dementia complex, is common in AIDS and is due to infection of the brain by HIV. We have tested the hypothesis that an HIV antigen suppresses neuronal responses to neurotrophic factors. These experiments utilize the preferential response of separate populations of embryonic chick dorsal root ganglion (DRG) neurons to either NGF or NLK. Neurons are grown at low density with either 1 nM NLK or 3 nM NGF in 16mm tissue culture wells coated with poly-L-ornithine and laminin. Cultures are microscopically scored for neuron survival after 48 hrs of growth. We find that: (1) HIV specifically inhibits the neurotrophic response of sensory neurons to NLK, but has no effect on the neurotrophic activity of NGF; (2) The inhibition is due to the external envelope glycoprotein of HIV (gp120); (3) A simian retrovirus related to HIV, STLV-III, also inhibits the response to NLK, but not to NGF, while another simian retrovirus unrelated to HIV, SAIDS-D, does not inhibit either NLK or NGF. We hypothesize that gp120 inhibits NLK due to sequence homology with the factor.

The homology with NLK is conserved in all isolates of HIV for which sequence information is available, and undoubtedly denotes strong selective pressure on HIV phenotypic expression. We now have experimental evidence which demonstrates the importance of this region for HIV infectivity. We find that a rabbit antiserum made against a synthetic peptide from the gp120 region of NLK sequence homology potently neutralizes HIV infectivity *in vitro*. In addition, recent studies from the laboratory of Dr. M. Martin (NIH) show that introduction of a single point mutation into a potential glycosylation site within the region homologous to NLK renders the mutant HIV noninfectious. However, neither the above peptide antiserum nor the point mutation decreases the ability of HIV to bind to CD4, the receptor for HIV on T lymphocytes. Though the region of NLK sequence homology is not involved with binding of HIV to CD4, it appears to be essential for HIV infectivity. In addition, we speculate that inhibition of neuronal response to NLK during HIV brain infection may occur, and therefore, have a role in the pathogenesis of the AIDS dementia complex.

465.12 INCREASED AUTOPHOSPHORYLATION OF CALMODULIN KINASE II AFTER TREATMENT WITH AN ORGANOPHOSPHORUS COMPOUND. E. Suwita*, D.M. Lapadula*, and M.B. Abou-Donia (SPON : M.M. Abou-Donia). Department of Pharmacology, Duke University Medical Center, Durham, N.C. 27710.

We have previously reported an altered Calmodulin Kinase II activity in brain cytosolic fraction of chickens 21 days after treatment with tri-o-cresyl phosphate (TOCP). TOCP is one of the organophosphorus compounds capable of inducing a delayed neurotoxicity (OPIDN or organophosphorus compound-induced delayed neurotoxicity). OPIDN is observed after a latent period of 1-2 weeks following a single administration of the compound. The condition is characterized by an ataxia which progresses to paralysis concurrent with a central-peripheral distal axonopathy. Although the pathogenesis of OPIDN has not been established, Ca^{++} - calmodulin dependent protein phosphorylation by Calmodulin Kinase II has been found to play an important role in the development of the neuropathy. In this report, the activity of Calmodulin Kinase II was further investigated in chickens given a single oral dose of 0 or 750 mg/kg and killed after 1 day following treatment. Brains were homogenized in 100 mM PIPES, pH 6.9, 10 mM EDTA and 10 mM EGTA (1 ml/g) and centrifuged at 100,000 xg for 1 hr. The cytosol from individual animal was passed through P-11 column which has been equilibrated with 30 mM PIPES, pH 6.9. The column was subsequently eluted with 150 mM and 350 mM NaCl in 30 mM PIPES, pH 6.9. The 350 mM NaCl fraction, which is enriched in Calmodulin Kinase II, was used for phosphorylation assays. Reactions were started by adding crude kinase from control or treated animals into a phosphorylation buffer of 10 mM PIPES, pH 7.4, 1 mM EDTA, 2 mM EGTA, 10 mM $MgCl_2$, 50 μM $CaCl_2$, 1 μg calmodulin and 5 μM [γ-^{32}P]ATP. Proteins were subjected to SDS-PAGE and autoradiography. The extent of phosphorylation was quantitated by using microdensitometry. The autophosphorylation of the three subunits of Calmodulin Kinase II was increased by 359%, 345% and 325% for α, β and β' subunit, respectively. The increase in autophosphorylation of Calmodulin Kinase II can also be mimicked in vitro by incubation of the purified kinase with diisopropyl phosphoro fluoridate (DFP), another OPIDN-causing compound. These data suggest that the autophosphorylation of Calmodulin Kinase II may be causally related to the development of the delayed neuropathy. Supported in part by NIOSH Grant No. OH02003 and NIEHS Grant No. ES02717.

465.13 NODAL AND PARANODAL STRUCTURAL CHANGES IN MOUSE OPTIC NERVE DURING WALLERIAN DEGENERATION. M. Hasegawa* and J. Rosenbluth. Departments of Physiology and Rehabilitation Medicine, New York University School of Medicine, New York, NY 10016.

Ultrastructural changes in the nodal and paranodal regions of myelinated mouse optic nerve fibers were studied during the course of Wallerian degeneration. Sixteen mice (Balb/c) were sacrificed 4 hr to 28 days after right-sided enucleation, and thin sections and freeze-fracture replicas of the left and right optic nerves were compared.

The first changes on the degenerating side, seen as early as 4 hr after enucleation, consisted of disorientation and segregation of axonal microtubules accompanied by accumulation of mitochondria, dense bodies and neurofilaments. In addition, focal axonal swelling was seen in internodal regions and at nodes. At 8 hr, abnormalities appeared in the paranodal axo-glial junctions, including retraction of terminal loops from the axolemma, disconnection of terminal loops from myelin lamellae and partial loss of transverse bands between terminal loops and the axolemma. These changes progressed to a maximum level on days 3-4, at which time markedly widened and swollen nodal regions were common. In some cases paranodes became greatly elongated as well, with wide separations between terminal loops, as if the inner loops had retracted from the nodal region and shifted in position toward the middle of the internode. Very short internodes were also sometimes encountered on degenerating axons. These changes did not progress uniformly in all fibers, and approximately one fourth of the myelinated fibers still remained morphologically intact on day 8. Even as late as day 28, intact fibers were still occasionally encountered among the degenerating axons and myelin debris.

In freeze-fracture replicas, the E- and P-faces of the nodal axolemma on day 3 showed a normal distribution of intramembrane particles, but the E-face of the paranodal axolemma showed partial loss and partial disorganization of the paracrystalline pattern normally seen there. Large particle-free areas within paranodes and immediately adjacent to nodes were visible on days 3-8, corresponding to regions of detached or retracted terminal loops seen in thin sections. The nodal axolemma displayed a patchy distribution of particles on day 8, but we could not follow changes in the node further because of axolemmal disintegration at later times.

In comparison with frog optic nerve undergoing Wallerian degeneration (J. Neurocytol., 15: 657, 1986), changes in the mouse follow approximately the same course, but proceed more rapidly. Furthermore, in the mouse, as in the frog, some of the fibers degenerate much more slowly than the majority, and some normal-looking fibers persist even at late stages.

Supported by grants from the NIH (NS-07495) and NMSS (RG-1579).

465.14 REGULATION OF MEMBRANE MEDIATED CHRONIC MUSCLE DEGENERATION BY DILTIAZEM, NIFEDIPINE, AND VERAPAMIL IN DYSTROPHIC HAMSTERS. Alice J. Crawford and Syamal K. Bhattacharya. Surgical Research Laboratories and Departments of Surgery and Medicinal Chemistry, University of Tennessee Medical Center, Memphis, TN 38163.

Excessive intracellular calcium accumulation has been shown to be a fundamental pathogenetic event associated with hereditary muscular dystrophy (Bhattacharya et al., Soc. Neurosci. Abstr., 12(1):264, 1986) and one of the earliest detectable biochemical changes in human fetuses at risk of Duchenne muscular dystrophy (Bertorini et al., Neurology, 34:1436-1440, 1984). We have also reported that oral administration of Diltiazem (DTZM), a potent Ca-antagonist, significantly reduced EICA and improved histopathologic lesions in the cardiac and skeletal muscles of BIO-14.6 strain dystrophic hamsers (DH) (Bhattacharya et al., Muscle & Nerve, 5:73-78, 1982).

Because of potential Ca-antagonistic properties of Verapamil (VPML) and Nifedipine (NFDN), we studied the relative efficacies of DTZM (25 mg/kg/d), NFDN (6 mg/kg/d), and VPML (25 mg/kg/d) in reducing EICA and improving the associated dystrophic pathobiology in 30-day-old male DH. These agents were administered orally for 180 days each to a group of 12 DH and 12 BIO-F1B strain normal hamsters (NH). Normal saline was given as a drug control to 12 DH and 12 NH. Plasma [Ca], [Mg], and CK activity, and tissue [Ca] and [Mg] of the heart, diaphragm, and rectus femoris muscles were quantitated. Histopathology of cardiac and skeletal muscles were also evaluated.

Data from this study congruently point to the overall relative efficacy of these agents in regulating excessive intracellular calcium accumulation in the following descending order: DTZM > NFDN > VPML. Muscle [Mg] was not significantly modified either by muscular dystrophy or by any one of the pharmacologic agents. In terms of the drug-toxicity and survival rate, we lost 5 DH and 2 NH on VPML, 3 DH on NFDN, 2 DH controls on normal saline during this investigation, but none on DTZM. Furthermore, NFDN produced significant edema in the hind limbs, soleus muscle, and myocardium.

In conclusion, Diltiazem appears to be the most effective agent in mitigating excessive intracellular calcium accumulation and chronic cellular degeneration in muscular dystrophy with the lowest incidence of toxicity and mortality. Further investigations of Diltiazem in combination with membrane stabilizing agents and α-/β- adrenergic regulators using improved drug delivery technique such as implanted osmotic minipumps are suggested.

(Supported by grants from Am. Heart Assoc., Muscular Dystrophy Assoc., and Varian Instrument Group of America).

465.15 DIRECT EVIDENCE OF MEMBRANE MEDIATED CELLULAR DEGENERATION BY CHANGES IN THE INTRACELLULAR FREE CALCIUM, POTASSIUM, AND SODIUM CONCENTRATIONS IN DYSTROPHIC HAMSTERS. S.K. Bhattacharya, J.R. Lopez*, V. Sanchez*, A.J. Crawford, J.L. Vergara*, and F. Sreter*. University of Tennessee, Memphis; Instituto Venezolano de Investigaciones Cientificas, Caracas; University of California, Los Angeles; and Boston Biomedical Research Institute, Boston.

We have shown that CHF-146 strain dystrophic hamsters (DH) represent an excellent model for the study of human forms of muscular dystrophy (MD) because of the striking pathogenetic similarities in the biochemical abnormalities, cellular necrosis, EKG changes, voluntary muscle weakness, and histopathology (Bhattacharya et al., Muscle & Nerve, 10:168-176, 1987). Our hypothesis that dystrophic muscle may acquire increased intracellular free [Ca] and recent findings of increased [Na] and decreased [K]$_i$ in muscle of DH led us to characterize intracellular free Ca ([Ca^{2+}]$_i$), Na ([Na^+]$_i$), and K ([K^+]$_i$) concentrations in the 75-day-old male DH and CHF-148 strain normal hamsters (NH).

Hamsters were anesthetized and the extensor digitorum longus (EDL) was removed by microdissection. [Ca^{2+}]$_i$ was measured using Ca^{2+}-selective microelectrodes (CSM) as previously reported (Lopez et al., Biophys. J., 43:1, 1983). CSM were calibrated before and after each series of measurements and only those which produced a linear response between pCa=3 and pCa=7 were used. The EDLs were mounted on a temperature controlled bath at 37°C and individual myofibers were impaled first with a 3 M KCl microelectrode to record the resting membrane potential and then with the CSM to measure the [Ca^{2+}]$_i$. Similarly, resting [Na^+]$_i$ and [K^+]$_i$ were determined in the EDL by ion selective microelectrodes prepared with liquid sensors based on the neutral ligand ETH-227 and potassium-ionophore (Valinomycin). Individual muscle fibers were impaled with two microelectrodes in order to measure simultaneously the resting membrane potential and the myofibrillar [Na^+]$_i$ and [K^+]$_i$.

The resting [Ca^{2+}]$_i$ in NH was 0.11 ± 0.01 uM (X ± SEM, N=35) while it was 0.43 ± 0.02 uM (N=48) in DH ($p<0.0001$). Likewise, [Na^+]$_i$ in NH was 8.5 ± 1.2 mM (N=10), while it was 16.4 ± 1.3 mM (N=10) in DH ($p<0.0001$). However, [K^+]$_i$ in NH was 114 ± 1 mM (N=15) and in DH was 103 ± 1 mM (N=14, $p<0.0001$).

These results suggest a generalized defect in the cellular ion-gating mechanism at the plasmalemmal level responsible for the regulation of intracellular free [Ca], [Na], and [K]. Our observations represent the first direct demonstration of significantly increased intracellular free [Ca] and [Na] with decreased [K] in the dystrophic myofibers and support our central hypothesis that alterations in these essential intracellular macro-electrolyte concentrations in MD are fundamental membrane mediated pathogenetic events. (Supported by Am. Heart Assoc.)

465.16 ABSENCE OF CALCIFIC DEGENERATION IN THE CARDIAC AND SKELETAL MUSCLE OF C57BL/6J-2J DYSTROPHIC MICE AND THEIR RELEVANCE AS A MODEL FOR MUSCULAR DYSTROPHY IN HUMANS. Debra J. Jackson*, Alice J. Crawford, and Syamal K. Bhattacharya (SPON: D.M. Desiderio). Surgical Research Laboratories, University of Tennessee, Memphis, TN 38163.

Because of hind limb deformity and neurogenic changes, C57BL/6J-2J dy/dy dystrophic mice (DM) have been studied for muscular dystrophy (MD) and Schwann cell abnormalities. In studies of CHF-146 strain dystrophic hamsters (DH) (Muscle & Nerve, 10:168, 1987), Davis line 412 dystrophic chickens (Soc. Neurosci. Abst., 12:264, 1986), and patients with Duchenne muscular dystrophy (DMD) (Neurology, 32:1088, 1982), we have shown that membrane mediated excessive intracellular Ca accumulation in cardiac and skeletal muscles is a fundamental pathogenetic event associated with genetic expression of chronic and progressive muscle degeneration in muscular dystrophy. Inherited membrane insufficiency produces elevated plasma CK, reduced muscle Mg concentration, and classical histopathology, in addition to profound EICA. We, therefore, studied these variables using the sensitive atomic absorption techniques of Bhattacharya et al. (Anal. Lett. 12:1475, 1979) in the cardiac and skeletal muscles of 8-mo-old male DM and compared with those obtained from age, sex, and strain matched normal (C57BL/6J-2J +/+) mice (NM). Plasma CK activity and muscle histology were also evaluated. Muscle mineral data (X ± SEM), mEq/kg fat free dry tissue, are given below.

	Myocardium		Rectus Femoris	
GROUP (N)	Ca	Mg	Ca	Mg
NM (8)	13.4 ± 0.8	87.6 ± 2.2	15.7 ± 0.5	90.1 ± 2.0
DM (7)	14.8 ± 0.9	83.2 ± 2.3	16.8 ± 0.9	86.9 ± 2.4
Significance	NS	NS	NS	NS

Above data are in contrast to our previous findings of 300% to 2000% increased muscle [Ca] and 20-45% reduced muscle [Mg] in DMD and DH in comparison to their normal counterparts. However, the soleus of DM has shown 40% excessive intracellular Ca accumulation and 18% [Mg] reduction (Bhattacharya et al., Clinical Res., 31:717, 1983). Cardiac tissue from DM did not reveal typical morphologic changes universally present in DMD or DH.

In conclusion, DM exhibit more neurogenic involvement than DH and some skeletal muscle impairments without involving the myocardium and calcific changes in the rectus femoris or diaphragm muscle, whereas DH reveal both the skeletal and cardiac muscle degeneration universally present in DMD patients. These results and the stricking similarities in the electrocardiographic abnormalities of DH and DMD strongly suggest that the DH model more closely resembles DMD than does the DM model. (Supported by grants from Am. Heart Assoc. and the Varian Instrument Group)

465.17 CHLORIDE AND POTASSIUM MEMBRANE CONDUCTANCES ARE DECREASED IN SKELETAL MUSCLE FIBERS FROM THE (mto) MYOTONIC MOUSE. S.H.Bryant, M.Mambrini* and R.K.Entrikin. Dept. Pharmacol. and Cell Biophys., Univ. Cincinnati, Cincinnati, OH 45267, Dipartimento Farmacol., Univ. Bari, Bari, Italy, and Dept. of Physical Medicine, School of Medicine, Univ. California, Davis, CA 95616.

The mto mouse strain first described by Heller et al. (J. Neurosci. 7:924, 1982) is an autosomal recessive mutant in which the homozygous mto/mto mice develop a nondystrophic myotonia with electromyographic, contractile and clinical abnormalities resembling myotonia congenita of man and the hereditary myotonia of goats. The heterozygous unaffected littermates (+/+ or mto/+) on the other hand show no clinical or EMG signs (Aichele, et al., Exp. Neurol. 87:377, 1985; Entrikin, et al., Muscle Nerve 15:111, 1987). Here we report experiments to test whether murine myotonia is associated with low chloride conductance as is caprine myotonia and human myotonia congenita. Standard microelectrode techniques were used to determine the cable parameters, resting component conductances, and excitability of single extensor digitorum longus (EDL) fibers from two myotonic (mto/mto) mice and two heterozygous nonmyotonic controls (+/?). Both EDL's were removed from each mouse (8 muscles total) and measurements were made in vitro at 30°C in a normal ringer or in chloride-free ringer (methylsulfate substitution) for separation of component conductances.

Total membrane conductance G_m, and component potassium G_k and chloride C_{Cl} conductances ($\mu S/cm^2$) are given in the table below (means ± SEM, N=number fibers):

	N	G_m	G_k	G_{Cl}
Myotonic (mto/mto)	23	297±28	251±37	46±25
Control (+/?)	41	3907±391	504±109	3403±312

Myotonic fibers show almost complete lack of chloride conductance. Potassium conductance was reduced 50%. When stimulated with long depolarizing pulses, myotonic fibers showed a propensity for repetitive firing and afterdischarge. Further, the myotonic fibers in normal ringer and the control fibers in low chloride ringer required TTX (10^{-6} M) in the bath to block myotonia when making cable measurements. In mathematical or drug simulations decreasing G_{Cl} to about 25% of normal is sufficient to induce myotonia. We suggest therefore that the absence of chloride conductance in the myotonic mouse is the principal cause of the myotonia. This disease model should prove very useful in the study of genetic control of ionic channels. (Supported by: NIH Grant NS-03178, MDA Grant-in-Aid, and U.S. Dept. Ed. Grant NIDRR #G008300078)

NEUROTOXICITY III

466.1 INACTIVATION OF γ-GLUTAMYLCYSTEINE SYNTHETASE AND GLUTAMINE SYNTHETASE BY OXYGEN FREE RADICALS: IMPLICATIONS FOR NERVOUS SYSTEM DAMAGE. Nina F. Schor* (SPON: P. Wade) Division of Child Neurology, Children's Hospital, University of Pittsburgh, Pittsburgh, PA 15213

Oxygen free radicals have been implicated in the toxicity of oxygen for the central nervous system and in post-ischaemic reperfusion injury seen after cardiac arrest and stroke. One physiologic mechanism for detoxification of oxygen radicals is their reduction by reduced glutathione. On the other hand, oxygen radical damage is exacerbated in the presence of the excitatory neurotransmitter, glutamate. We present herein evidence for the inactivation of enzymes responsible for the synthesis of glutathione and for the conversion of glutamate to its inactive metabolite, glutamine, by oxygen radicals.

It has long been thought that oxygen radicals deplete cellular glutathione by oxidizing it to its disulfide form. However, we have found that total (reduced plus oxidized) glutathione levels decrease in A/J mice given 6-hydroxydopamine, an oxygen radical generator. This implies that oxidation of reduced glutathione is not the sole mechanism of its depletion. We have recently found that the rate limiting enzyme for glutathione synthesis, γ-glutamylcysteine synthetase, is inactivated by 6-hydroxydopamine at concentrations of the drug which are theoretically obtainable in animals. Inactivation of the synthetase by 0.67mg/ml 6-hydroxydopamine is complete by 2 hours at pH 7.0 and 37°C. The inactivation is not reversible by dialysis or by reduction with dithiothreitol, and is greatly attenuated in the presence of superoxide dismutase.

Glutamine synthetase is an enzyme with a binding site for glutamate which is structurally similar to that of γ-glutamylcysteine synthetase. This enzyme provides a major mechanism for inactivation of glutamate in the central nervous system. We have found that glutamine synthetase is completely inactivated by incubation with 6-hydroxydopamine. This inactivation has the same time course as that of γ-glutamylcysteine synthetase and is also attenuated in the presence of superoxide dismutase. That these enzyme inactivations are somewhat specific is shown by our finding that the activity of glutathione reductase is not altered by incubation of this enzyme with 6-hydroxydopamine.

These findings indicate that oxygen radicals may perpetuate their own toxic effects upon the nervous system by paralyzing their own detoxification and by prolonging cellular exposure to glutamate.

(This work was supported by the Pittsburgh Cancer Institute and by the Department of Pediatrics, University of Pittsburgh.)

466.2 DEPRESSION OF THE MONOSYNAPTIC REFLEX IN THE NEONATAL RAT SPINAL CORD BY DIISOPROPYLPHOSPHOROFLUORIDATE AND ITS REVERSAL BY ATROPINE. Shyamal Das Gupta* and Jordan E. Warnick (SPON: N.S. Pilotte). Dept. of Pharmacol. & Exptl. Ther., University of Maryland School of Medicine, Baltimore, MD 21201.

Diisopropylphosphorofluoridate (DFP) is a organophosphorus (OP) compound which is known to have multiple actions. In addition to inhibiting acetylcholinesterase, with the consequent elevation of acetylcholine levels at both central and peripheral cholinergic sites, DFP also blocks the ion channel of the nicotinic acetylcholine receptor (Kuba and Albuquerque, *Science* 181:853, 1973). Recent work in this laboratory has shown that sarin, an irreversible OP agent, facilitated the monosynaptic reflex at 5-20 nM and reduced frequency-dependent depression in isolated neonatal spinal cords in which acetylcholinesterase had previously been inhibited by DFP (Yang & Warnick, *Fed. Proc.* 43:929, 1984). We now report that DFP depresses the monosynaptic reflex in the neonatal spinal cord, an action which is antagonized by atropine. Spinal cords were removed from 6- to 8-day old female rats, hemisected, placed in an experimental chamber and superfused with physiological solution at 25 °C. A monosynaptic reflex was evoked by stimulating the L_3 or L_4 dorsal root and recorded from the corresponding ventral root.

DFP caused a concentration-dependent depression of the monosynaptic reflex. At 100 nM, DFP had no effect when applied for 15 min, but caused 8, 33, 54 and 70% depression of the monosynaptic reflex at 1, 10, 100 and 500 μM, respectively, while enhancing frequency-dependent depression. Recovery from DFP-induced depression was dependent upon the concentration of DFP: the higher the concentration, the longer the washing period before recovery. For example after exposure to 3 μM DFP, recovery after washing occurred in 15-20 min while at 100 μM, it took about 2 hours. The depression caused by DFP could be completely and quickly (<10 min) antagonized by exposure to atropine (500 nM). A lower concentration of atropine (30 nM) was equally effective in preventing the depression caused by DFP (10-500 μM). The depression of the monosynaptic reflex by DFP was unrelated to inhibition of acetylcholinesterase and is similar to that caused by the cholinergic agonists carbamylcholine and oxotremorine (Swanson and Warnick, *Abs. Soc. Neurosci.* 10:417, 1984). The beneficial action of atropine in DFP-induced depression may be attributed to the protection of a cholinergic receptor or mediated by a reduction of presynaptic stores of bound acetylcholine. DFP's action may therefore result from blockade of a cholinergic receptor. (Supported by U.S. Army Medical Research and Development Command Contract DAMD17-85-C-6030.)

466.3 PROLONGED EFFECTS OF DFP ON THE NEUROCHEMISTRY AND ELECTROPHYSIOLOGY OF THE NEOSTRIATUM IN RATS.
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Exposure to organophosphates in humans results in both acute and chronic neurological aftereffects. Prominent among these sequelae are movement disorders whose nature is only partially consistent with the spinal lesions known to be induced by organophosphates. Rather, certain of these symptoms suggest that forebrain motor areas may also be damaged. This study was an initial attempt to investigate this suggestion. Attention was focussed on the neostriatum because of its known involvement in motor control and its high acetylcholine (ACh) concentration.

In neurochemical experiments, rats were dosed with 4mg/kg DFP s.c. and either killed 90 minutes later or given atropine (20mg/kg i.m.) at the time of injection and killed 2 weeks later. Neostriatal AChE was inhibited more than 95% at 90 minutes. For neurotransmitter determinations, rats were killed by head-focussed microwave irradiation. ACh and catecholamines were determined by HPLC with electrochemical detection. Neostriatal ACh levels were elevated at both times after DFP administration. While neostriatal dopamine (DA) levels were not significantly altered, DOPAC levels and DOPAC/DA ratios were elevated at both times. These changes indicate decreased and increased activities, respectively, of cholinergic and dopaminergic neurons.

Changes were observed in the electrophysiology of the neostriatum that were in accord with the altered neurochemistry. Field potentials were recorded in the neostriatum in response to activation of a relatively direct input (electrical stimulation of the somatomotor cortex) and a polysynaptic pathway (electrical stimulation of the forepaw). In 2-week DFP rats, both types of field potentials were reduced in amplitude and were unable to follow repetitive stimulation at rates greater than 20Hz. These effects were most marked in the potentials evoked by stimulation of the forepaw. In addition, the latter responses showed abnormally rapid habituation.

In conclusion, DFP causes persistent changes in the neurochemistry and electrophysiology of the neostriatum. Whether these changes are manifestations of a direct influence or a reflection of changes in strucures afferent to the neostriatum remains to be determined.

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466.4 CORRELATION OF NEUROPATHY TARGET ESTERASE ACTIVITY WITH A SPECIFIC TRITIATED DIISOPROPYLFLUOROPHOSPHATE LABELED PROTEIN.
T. C. Thomas*, M. G. McNamee and B. W. Wilson. Dept. of Biochem. and Biophys. and Dept. of Avian Sci., Univ. of Calif., Davis, CA 95616.

Neuropathy Target Esterase (NTE) is an integral membrane carboxylesterase found in central and peripheral nerve tissues. Its inhibition by certain organophosphates, such as DFP and Mipafox, is highly correlated with the ability of these compounds to cause organophosphate ester induced delayed neuropathy (OPIDN). We are investigating the possibility that NTE is the target for initiation of this delayed neuropathy. The following work extends the approach used by Ishikawa et. al. (1) and Wilson et. al. (2). NTE was identified by its sensitivity to inhibition by Mipafox and resistance to inhibition by Paraoxon (a non-neuropathic inhibitor). Gel filtration of high salt, Triton X-100 solubilized proteins from chicken brain microsomes using Sephacryl S300 separated four distinct classes of phenyl valerate hydrolyzing carboxylesterase activity. It was determined by this method that NTE has a molecular weight of at least 1.5×10^6 daltons. Solubilized membrane proteins were fractionated on 5-20% sucrose density gradients, and NTE was found in a 9S peak while the majority of esterase activity was found in a distinct 4S peak.

In a second set of experiments microsomal membranes were labeled with ^{3}H-DFP in the presence and absence of mipafox and/or paraoxon. Electrophoresis of labeled membranes on 7.5% SDS-PAGE under reducing conditions identified a major 160 kd band and a minor 80 kd doublet as candidates for NTE. When a high salt, Triton X-100 extract of labeled membranes was centrifuged on a sucrose density gradient the paraoxon-resistant mipafox-sensitive label was found exclusively in the 9S peak. We have isolated this labeled peak and subjected it to SDS-PAGE. The 9S peak contained a single labeled protein of 160 kd which displayed a sensitivity to inhibition of labeling matching the inhibitor sensitivity of NTE. These experiments indicate that NTE can be separated from the other classes of carboxylesterases found in neural membrane preparations, and that NTE enzyme activity isolated from sucrose gradients correlates well with the ^{3}H-DFP labeled 160 kd protein. Comparison of mobilities determined by gel filtration and gel electrophoresis indicates that NTE may be a multi-subunit enzyme or part of a multi-enzyme complex. We are continuing efforts to purify and characterize this enzyme in order to determine its role in the onset of delayed neuropathy. (This work was supported in part by NIH Grant ES00202; T.C.T. is a trainee on NIH Training Grant GM07377.)

1. Ishikawa, Y., Toxicology Letters, 17:315, 1983.
2. Wilson, B. W., in "Cholinesterases, Fundamental and Applied Aspects", Walter de Gruyter, New York, 1984, pp. 493-506.

466.5 COMPARISON OF HYPOXIA AND SOMAN INDUCED FINE STRUCTURAL CHANGES IN RAT HIPPOCAMPAL SLICE CA3 PYRAMIDAL NEURONS.
K.C. Sikora-VanMeter, W.G. VanMeter, R.C. Wierwille* and F.J. Lebeda. Dept. Pharmacol. and Exptl. Therap., School of Med., Univ. of MD, Baltimore, MD 21201; Dept. Physiol. and Pharmacol., Col. Vet. Med., Iowa State Univ., Ames, IA 50011; Dept. Neurol., Sect. Neurophysiol., Baylor College of Medicine, Houston, TX 77030.

Rat hippocampal slices were incubated in control saline for 60 minutes prior to exposure to pinacolyl methylphosphonofluoridate (soman), or hypoxia (95% N_2 : : 5% O_2). Electrical activity was recorded and the slices were prepared for electron microscopic analysis.

Fine structure in control slices during the 60 minute incubation in control saline after slice preparation includes, dilated rough endoplasmic reticulum (rER) in 70% of the CA3 neurons and slight swelling of mitochondria. Clumping of heterochromatin occurs in 10% of these cells. Moreover, the CA3 neurons show a continued recovery from the shock of slice preparation if incubation is continued beyond 60 minutes.

Application of 100nM soman (15-180 minutes) reduces acetylcholinesterase activity to less than 30% of control values and initiates periodic epileptiform electrical events in the CA3 subfield. The number of CA3 neurons showing indentations in the nuclear membrane increases in a time dependent manner. The Golgi complexes are highly developed and rER is proliferated. Moreover, these morphological changes are comparable to those in whole animals exposed to subconvulsant doses of soman.

In contrast, hypoxia (15-180 minutes) induces dilation of the rER in CA3 neurons together with time dependent partial or complete depletion of cytoplasm as well as extreme damage to nuclear chromatin and mitochondria. Therefore, these data do not support the hypothesis that soman induced alterations of CNS tissue are caused by hypoxia.

In conclusion, acute fine structural soman induced alterations in vitro are comparable to those seen after in vivo intoxication with subconvulsant doses and are unrelated to the neuropathogenic responses to hypoxic conditions.

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466.6 DEGENERATION OF THE MOSSY FIBER ENDINGS IN THE HIPPOCAMPUS OF NEAR-TERM RHESUS FETUSES TREATED PRENATALLY WITH DEXAMETHASONE.
H. Uno, S. Dong*, M.J. Engle* and J.W. Kemnitz. Wisconsin Regional Primate Research Ctr., Dept. of Pathology and Laboratory Medicine, and Dept. of Pediatrics, Univ. of Wisconsin, Madison, WI 53715.

Our previous studies revealed marked depletion and hypoplasia of the dentate granular and CA3 pyramidal neurons in the hippocampus of near-term (162 days of gestation) rhesus fetuses treated with dexamethasone (1.25 mg/kg mother's body weight, 4 times) at 132 to 133 days of gestation. Poor development of the stratum lucidum in the CA3 region was also noted. To observe the sequential changes of synaptogenesis of the mossy fiber terminals in the dendritic branches of CA3 pyramidal neurons, we studied the ultrastructure of the dentate granular and CA3 pyramidal neurons and synaptic terminals in the stratum lucidum of CA3 region in dexamethasone-treated 162-day fetuses. The ultrastructure of normal synaptogenesis was observed in the hippocampus of vehicle-treated 135-day as well as untreated 160- and 162-day fetuses. Immediately after delivery by cesarean section, fresh brain slices of hippocampus gyrus were cut and fixed overnight with a routine EM fixative at 4°C. After fixation, small tissue blocks (1 x 2 x 1 mm) containing the dentate and CA3 region (including the stratum pyramidal and lucidum) were dissected and prepared for EM procedures. In untreated hippocampus of near-term fetuses, a large number of the mossy fiber endings containing many spherical synaptic vesicles and mitochondria were observed in the stratum lucidum, and the endings formed synaptic contact with the dendritic spines of CA3 pyramidal neurons. The stratum lucidum was densely packed with dendritic branches of the pyramidal neurons and numerous preterminal axons and synaptic endings of the granular cell axons (mossy fibers). Both dentate granular and CA3 pyramidal neurons were well developed and their perikarya contained Nissl bodies, mitochondria, and ribosomes. In dexamethasone-treated fetuses, virtually all synaptic endings and preterminal axons of the mossy fibers in the stratum lucidum showed marked swelling and the synaptic vesicles, mitochondria, and neurotubules dispersed in edematous axoplasm. The dendritic branches of CA3 pyramidal neurons also showed edematous swelling and occasionally a few axo-dendritic synapses were found. Both dentate granular and CA3 pyramidal neurons showed atrophic perikarya containing many swollen mitochondria, dispersed ribosomes, and dilated endoplasmic reticulum. Dexamethasone treatment during the mid-third trimester caused persistent degenerative changes in the mossy fiber endings and hypoplastic changes of the granular and pyramidal neurons in the hippocampus of rhesus monkeys. (Supported by NIH grants HL27358 and RR00167.)

466.7 EFFECT OF CHRONIC CORTICOSTERONE ADMINISTRATION ON THE SEPTO-HIPPOCAMPAL CHOLINERGIC SYSTEM OF THE RAT. Y. Tizabi, V. H. Gilad* and G. M. Gilad. Dept. of Pharmacology Howard Univ. Washington, D.C. 20059 and Center for Neurosci. & Behav. Res. The Weizmann Inst. of Science Rehovot, Israel.

Degeneration of the septo-hippocampal cholinergic system is considered to be critically involved in aging and dementias. Glucocorticoids have been shown to be capable of accelerating the degeneration of hippocampal neurons. Since the hippocampus receives an extensive cholinergic input from the medial septum, it was of interest to examine the effect of glucocorticoids on this input. Nine adult male rats were injected i.p. once daily, six days a week for 2 months with 10 mg of corticosterone in peanut oil. Controls received vehicle injection. Twenty four hours following the last injection 3 rats from each group were anesthetized and perfused for histological studies. The remaining rats were decapitated and the septum, hippocampus, frontal cortex and striatum were dissected free. ^{3}H choline uptake was not affected in the hippocampus or the striatum. Corticosterone caused a 30% increase in newly synthetized ^{3}H-acetylcholine release in the striatum but not in the hippocampus. Choline acetyltransferase (CAT) activity was reduced in the striatum (15%) and the septal area (20%) with no change in the hippocampus or the frontal cortex. ^{3}H-Quinoclinidyl benzylate (QNB) binding was reduced by 50% in the hippocampus and 28% in the striatum with no change in the frontal cortex. The number of acetylcholinesterase (AChE)-stained neurons in the medial septal area was reduced by about 30%, but no apparent cell loss was detected in the hippocampus utilizing cresyl-violet staining. Previous data indicate that 3 months of corticosterone treatment can cause pyramidal cell loss in the hippocampus (Sapolsky, et al. J of Neurosci. 5:122, 1985). The present data indicate that 2 months of corticosterone treatment causes cell loss in the medial septal area but not in the hippocampus. We conclude that corticosterone, the major glucocorticoid of rats, causes a spatio-temporal effect on the septo-hippocampal system with septal cholinergic cells being affected prior to their target area. Furthermore, we postulate that a failure to find a decrease in CAT activity in the hippocampus, despite an apparent loss of septo-hippocampal cholinergic cells may reflect a compensatory response in surviving cell terminals.

466.8 ANATOMICAL AND PHYSIOLOGICAL EFFECTS OF SYSTEMICALLY ADMINISTERED KAINIC ACID IN THE RAT CENTRAL NERVOUS SYSTEM. M.Thomas* and R.B. Wallace.Developmental Psychobiology Lab.,Univ. of Hartford, West Hartford, Ct. 06117.

Systemic administration of Kainic Acid (KA) produces selective neuronal degeneration in discrete areas of the olfactory system, amygdala,hippocampus,thalamus and neocortex.

KA belongs to a closely related group of acidic amino acids which possess a bifunctionality.Termed the Excitotoxins by Olney et al. (Brain Res.:77,1974),each of these compounds has the ability to cause both excitation and damage to nerve cells.Originally,it was thought that KA had specificity for glutamate (Glu) receptors.The increased Glu receptor stimulation elicited continual excitation resulting in Na^{+} and K^{+}build-up beyond the corrective abilities of membrane pumps.The severe change in gradients would,thus,create an enviroment incompatible with cell function and cell degeneration would ensue.

The basic hypothesis of the KA mechanism has not changed;damage is still attributed to excessive depolarization.The specifics on how KA causes the depolarization,however,has been the focus of many changes.

Johnston et al. (J.Neurochem.:32,1979) demonstrated through radioactive binding and differential sensitivity studies that KA does not activate the majority of Glu receptors,but rather only the "folded" L-Glu receptor.It has been shown recently from in-vitro autoradiographic binding studies that KA binding receptors are localized in discrete areas of the CNS.The greatest density of receptors is seen in the region corresponding with the zone of termination of mossy fibers on CA_3 pyramidal cells of the stratum incidum (Foster,Mena,Monaghan and Cotman,Nature:289,1981).When this same area was tested for Glu binding there appeared to be none, which suggests the presence of a KA-specific receptor.

Reproducibility of the damage patterns was tested by the following experiment.Six Long-Evans Hooded rats were selected from our colony and divided into two groups (A and B).Animals of both groups received an injection (ip) of KA at doses of 5,7.5 or 12 mg/kg,respectively.Group A was allowed to survive 4 hrs and group B 48 hrs. Each animal was sacrificed by intracardial perfusion.The brains were extracted after perfusion and post-fixed in formalin/$KClO_3$.All tissue was stained by the Marchi method (Humason,Animal Tissue Techniques,3rd ed.,1972).

Sections were cut at 8μm and viewed by light microscopy.The results obtained support the previous findings which suggest a KA-specific receptor localized in KA sensitive terminal fields.The Marchi stain allowed for the identification of not only general area effects,but specific structure sensitivity as well.Neocortical layers V and VI,fimbria hippocampi,olfactory tubercule and lateral olfactory tract are among the most sensitive areas to KA toxicity.

466.9 KAINIC ACID LESIONS OF THE LATERAL GENICULATE NUCLEUS: ALTERED COURSE OF AXON TERMINAL DEGENERATION FOLLOWING ENUCLEATION. C.K. Meshul, W.R. Woodward and L.M. Lund* V.A. Medical Center and Dept. Neurol., Oregon Health Sciences Univ., Portland, OR 97201.

It has previously been shown that kainic acid (KA) injections in rat dorsal lateral geniculate nucleus (dLGN) completely destroy geniculate neurons but does not affect axons from the retina or visual cortex which terminate in or pass through this nucleus (Woodward and Coull, 1982). Retrograde transport studies of fluorescent dyes or HRP injected into KA lesioned dLGNs showed that the nerve terminals of cells originating in either the retina or visual cortex were functional. Electron microscopic studies revealed the presence of small, medium and large axon terminals, which appeared intact and filled with synaptic vesicles. Many of these terminals appeared to make synaptic-like contact with other axons, with astrocytes and with postsynaptic membrane fragments of indeterminate origin. In order to identify those terminals projecting from the retina, we enucleated animals with KA lesions and looked for ultrastructural evidence of axon terminal degeneration. Control and KA lesioned animals were enucleated, perfused after 1, 3, 7, 15 and 30 days, and dLGNs were processed for electron microscopy.

In control animals degenerating retinal terminals showed characteristic darkening of the cytoplasm and clumping of synaptic vesicles. Two distinct waves of degeneration were observed in small and medium sized terminals: the first wave occurring by 1 day after enucleation and the second by 15 days. Large terminals showed evidence of degeneration between days 3 and 7 post-enucleation, and no degenerating profiles were observed at day 30. In contrast, KA lesioned geniculates did not show the darkening of axon terminal cytoplasm and clumping of synaptic vesicles or other signs of degeneration, even up to 30 days following enucleation.

One explanation for the differences in response to axotomy between lesioned and control dLGNs is that KA may alter axon terminals. For example, in KA-lesioned geniculates the rate of terminal degeneration may be significantly slowed, or ultrastructural signs of degeneration may be different. Alternatively, removing target neurons or altering glial cells in dLGN may influence degeneration in retinal ganglion terminals either by altering the metabolic state of these endings or by eliminating trophic influences. This situation may be similar to that seen in hibernating ground squirrels in which distal segments and terminals of transected motor nerves do not degenerate (Albuquerque, Deshpande and Guth, 1978). One approach which might allow us to distinguish between these possibilities would be to provide new targets for retinal ganglion terminals by transplanting fetal thalamic tissue into lesioned geniculates.

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466.10 **EXCITATORY AMINO ACIDS DO NOT CAUSE DEATH OF MAMMALIAN RETINAL GANGLION CELLS IN CULTURE.** J.S. Hahn*, E. Aizenman, S.A. Lipton (SPON: S. Fischel). Program in Neuroscience, Dept. of Neurology, The Children's Hospital, Harvard Medical School, Boston, MA 02115.

Excitatory amino acids (EAAs) have been implicated in neurotoxicity in several central nervous system preparations. In fact EAA neurotoxicity was possibly first described in the inner layer of the mouse retina following systemic injections of glutamate (Lucas and Newhouse *Arch. of Ophthalmology* 58:193, 1957). Since then, the effects EAAs on the vertebrate retina have been studied repeatedly but sometimes with conflicting results regarding toxicity to retinal ganglion cells (RGCs; Morgan & Ingham, *Neurosci. Lett.* 21:275, 1981; Goto et al., *Brain Res.* 211:305, 1981). In the present study we looked further into this question by studying the effects of EAAs on RGCs in culture. EAA responses have been recorded from solitary RGCs in our laboratory using patch clamp techniques, and the phamacologic properties of cultured RGCs to EAAs have been characterized (E. Aizenman et al., *Soc. Neurosci. Abstr.* 12:57, 1986; Karschin et al., *Ibid,* 1987). RGCs from 4-7 day old rats were labeled by retrograde transport of the fluorescent dye granular and maintained in dissociated cultures (Leifer et al. *Science* 224:303, 1984). After a 6 hr incubation, the cells were treated with several EAAs at varying concentrations. A set of sibling cultures was treated in the same manner but in the absence EAAs. After a further incubation of 12-24 hr, the viability of RGCs was scored using fluorescein diacetate.

Treatment of cultures with 125 μM kainic acid (KA) produced no significant increase in RGC death compared with controls. Neither solitary or clustered RGCs were affected despite the fact that about half of the clustered RGCs have synaptic inputs in our cultures (Lipton, *PNAS* 83:9774, 1986). This result seemed somewhat surprising in light of the fact that kainate (50-125 μM) produced non-desensitizing electrical responses in 100% of the RGCs in culture. Similarly, 200 μM N-methy-D-aspartate (NMDA) alone or in combination with 125 μM KA did not increase RGC death. The RGCs were also treated with glutamate (0.5, 1.0, and 5.0 mM). Even at these high doses, there was no significant decrease in the number of living RGCs after 12-24 hours of treatment compared to controls. Exclusion of magnesium from the growth medium also did not produce toxicity in cultures treated with 200 μM NMDA or 500 μM glutamate. Since NMDA-induced electrical responses even in the absence of Mg^{++} are generally small (170-670 pS), it remains possible that the lack of cell death in our system reflects a low density of NMDA receptors. This study indicates that the presence of electrophysiological responses to EAAs is not sufficient for EAA-induced neurotoxicity in cultures from the postnatal mammalian CNS.

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466.11 AN EXCITATORY AMINO ACID ACTIVATES CALPAIN I AND STRUCTURAL PROTEIN BREAKDOWN IN VIVO. J.C. Noszek* and R. Siman, Neuroscience Group, Medical Products Dept., The DuPont Co., Wilmington, DE. 19898.

Mammalian brain contains two calcium-activated proteases, calpain I and calpain II, that are activated, respectively, at low micromolar and high micromolar calcium concentrations. Calpain activation has been hypothesized to be critically involved in structural modification of synapses, and in neuronal degeneration. It has not yet been demonstrated, however, that physiological stimuli can activate the calpains in vivo. We report here that administration of the excitatory amino acid kainate in vivo causes activation of calpain I and degradation of neuronal structural proteins.

Rats were administered kainate (12 mg/kg) intraperitoneally and allowed to survive for 1,3, or 6 hours. The extent of calpain activation was assessed in dorsal hippocampus, taking advantage of the property of the calpains to undergo autoproteolysis upon activation. Calpains I and II were separated by SDS-PAGE and detected and quantified by immunoblotting with polyclonal antibodies to the Mr~84kD catalytic subunit of human erythrocyte calpain I. Blots of partially purified rat brain calpain I or rat brain calpain II indicated that the antibodies detect the catalytic subunits of both proteases (rat brain calpain I Mr~84kD, calpain II Mr~76kD). Kainate induced a time-dependent decrease in calpain I but had little effect on calpain II, with calpain I levels decreasing to 55% of control by 6 hours. Concomitant with the calpain I decrease, kainate stimulated the degradation of brain spectrin and the Mr~200kD neurofilament subunit, quantified by immunoblotting with appropriate antibodies. Spectrin proteolysis was accompanied by a four-fold increase in two lower molecular weight breakdown products; these fragments are of identical size as those produced upon cleavage of purified brain spectrin by purified calpain I. In contrast to the spectrin and neurofilament polypeptides, kainate did not affect glial fibrillary acidic protein, an excellent calpain substrate in vitro, and did not alter levels of actin, a poor calpain substrate.

These results indicate that excitatory amino acids can provide sufficient intracellular calcium to activate the high-sensitivity protease calpain I, without apparently affecting the low-sensitivity variant, calpain II. The activation occurs in neurons, but perhaps not in glia, and leads to degradation of major neuronal structural proteins. The findings support the hypothesis that calpain I activation is an obligatory step in the neurotoxic action of excitatory amino acids. Conceivably, less pronounced stimulation of excitatory amino acid receptors than employed here could act through calpain I to produce more modest structural changes than those associated with neurotoxicity.

466.12 EXCITATORY AMINO ACID-INDUCED NEUROTOXICITY: BLOCKADE BY PROTEASE INHIBITORS. R. Siman, C. Brady* and J.P. Card (SPON: J. Kauer). Neuroscience Group, Medical Products Dept., The DuPont Company, Wilmington, DE. 19898.

Sustained stimulation of receptors for excitatory amino acids (EAAs) leads to the death of receptive neurons. Because this form of neurotoxicity has been proposed to be involved in the degeneration associated with a number of neuropathologies, the underlying molecular mechanisms are of considerable interest. Studies of in vitro model systems have pointed to depolarization-induced influxes of either calcium or chloride as the trigger. We have been using the hippocampal slice as a preparation amenable to molecular study; in this preparation, EAAs elicit acute neuronal damage that shares several properties with EAA neurotoxicity in vivo. Here, we have examined whether calcium influx triggers the hippocampal damage and, if so, what subsequent intraneuronal event calcium activates to initiate the toxic process.

Transverse hippocampal slices were prepared from adult rats and maintained by conventional methods. Slices were embedded in plastic, sectioned at 1 um, stained with toluidine blue, and examined by light microscopy. Compared with untreated slices, slices incubated for 1.5 hours with 30-100 uM kainate or N-methyl-D-aspartate (NMDA) exhibited severe pathology. Nearly all pyramidal and dentate granule neurons showed extreme swelling, cytoplasmic and nucleoplasmic disintegration, and plasma and nuclear membrane ruffling and collapse. Numerous small (<5um) unstained profiles filled the neuropil. Electron microscopy identified them as massively swollen pathologic dendritic shafts and spines. Based on selective antagonism by DL-AP7, NMDA exerted its toxicity by stimulating a receptor class distinct from that stimulated by kainate or quisqualate. Quisqualate was significantly less potent as a neurotoxin than either NMDA or kainate. All three agonists required calcium to be neurotoxic, as removal of extracellular calcium protected both perikarya and dendrites. Since hippocampal neurons contain protein-degrading enzymes activated by calcium, the calpains, inhibitors of proteases such as the calpains were tested for their effect on EAA neurotoxicity. Two protease inhibitors of broad specificity, leupeptin and antipain, and one specific for thiol-proteases, E-64 (at 200 uM), reduced the acute damage produced by kainate, NMDA, or quisqualate. Both neuronal somata and dendritic processes were protected.

Therefore, chronic stimulation of EAA receptors damages hippocampal neurons by mechanisms that include a calcium-dependent step. Furthermore, protease activation is required for toxicity. Coupled with the recent finding (Noszek et al., this meeting) that EAAs can activate the calcium-dependent thiol-protease calpain I in vivo, the results suggest that calpain I activation may be necessary for the expression of EAA neurotoxicity.

466.13 CAPSAICIN INDUCES NEURONAL DEGENERATION IN MIDBRAIN AND FOREBRAIN STRUCTURES IN NEONATALLY TREATED RATS. T.T. Dinh* and S. Ritter (SPON: F. Young). College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6520.

Capsaicin is a neurotoxin that causes degeneration in brain and spinal cord of both neonatal and adult rats. The prevailing view is that this degeneration is limited to areas of the spinal cord and caudal hindbrain that receive primary afferent input. However, using a cupric silver stain for labeling degenerating neurons, we recently found that systemic capsaicin treatment of adult rats caused degeneration in discrete brain loci not traditionally associated with primary sensory innervation. Despite the fact that neurotoxicity of capsaicin is assumed to be greater in neonates than in adults, degeneration in those novel sites has not been reported in neonates. Therefore, we reexamined the effect of capsaicin on the central nervous system of the neonatal rats using silver stain techniques.

Sprague-Dawley rats were injected with a single dose of capsaicin (50 mg/kg, s.c.) or capsaicin vehicle solution (10% alcohol:10% Tween in saline) at 2 or 10 days of age and sacrificed 6, 12 or 24 hrs later. Processing with a modified Carlsen-deOlmos cupric silver stain revealed that capsaicin-induced degeneration was present in areas known to be recipients of primary afferent input: the spinal cord dorsal horn, spinal trigeminal nucleus, the solitary nucleus and the lateral borders of the area postrema. Degeneration was also present in all of the remaining areas in which we recently found degeneration in adult capsaicin-treated rats: the inferior olivary nucleus (n.), the olivary pretectal n., the interpeduncular n., the suprachiasmatic n., and the lateral septal/medial accumbens region. In addition, degeneration was observed in several areas not reported previously for either neonates or adults: the supramammillary n., the lateral geniculate n., the dorsal tegmental n. and a region in the dorsal thalamus. Finally, in 2-day-old rats, but not in 10-day-old rats, the vehicle solution alone caused degeneration. The latter was most prominent in cerebellar fiber pathways.

These results reveal that degeneration is more widespread in the brain after neonatal capsaicin treatment than observed previously in either adult or neonatal rats. It occurs both in sites which are known recipients of primary afferent neurons and in sites where primary afferent input has not been described. Thus, it is possible that a small and select population of brain neurons have characteristics similar to primary sensory neurons, which makes them sensitive to capsaicin's neurotoxicity.

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467.1 REVERSE TRANSCRIPTASE ACTIVITY IN PERIPHERAL LYMPHOCYTE CULTURES OF PATIENTS WITH SCHIZOPHRENIA AND NORMAL CONTROLS. A. Feenstra*, D.G. Kirch*, M.A. Coggiano*, D. Venable* and R.J. Wyatt (SPON: J.R.Stevens). Neuropsychiatry Branch, NIMH, Washington, D.C.

The evidence that schizophrenia may involve infection by a virus (or viruses) has been indirect. This evidence includes the phenomenology of schizophrenia (insofar as it may be mimicked by some viral encephalitides), epidemiological factors (including a predominance among patients with late winter/early spring births, a north-south gradient, and occasional clustering of cases), and indirect laboratory evidence (gliosis in some neuropathological studies, spinal fluid protein abnormalities, and abnormalities in cell-mediated immunity).

The discovery of the human retroviruses, HTLVI, HTLVII and HIV, now also known to affect the CNS, together with the development of new techniques in human retrovirology made it possible to investigate the role of this class of viruses in the etiology of schizophrenia.

From 20 chronic schizophrenic patients and 10 normal subjects short term tissue cultures of peripheral lymphocytes were established. The cells were grown in the presence of T-cell growth factor, and the culture supernatant was tested for the presence of reverse transcriptase every three to six days. Under these conditions no T-cell associated reverse transcriptase activity was detected in our cultures from patients or normals. To investigate the possibility that a retrovirus is present, but not activated under these conditions, we treated the cells with 2.5-5 uM 5-Azacytidine. This agent is known to affect viral gene expression by altering the extent of DNA-methylation. In initial experiments no reverse transcriptase activity could be detected in the 5-Azacytidine treated cultures. Further studies are necessary however to substantiate this finding.

In conclusion, we found no T-cell associated reverse transcriptase in peripheral lymphocyte cultures of our patients. Therefore, these data do not support an association of a T-cell lymphotropic retrovirus with schizophrenia.

467.2 RECANALIZATION FOLLOWING RAT MIDDLE CEREBRAL ARTERY THROMBOTIC OCCLUSION: HISTOPATHOLOGICAL, HEMODYNAMIC AND MICROVASCULAR CONSEQUENCES. H. Nakayama, W.D. Dietrich, B.D. Watson, R. Busto, and M.D. Ginsberg (Spon: (R.G. Clark). Cerebral Vascular Disease Research Center, Departments of Neurology and Anatomy and Cell Biology, University of Miami School of Medicine, Miami, FL 33101.

Human thromboembolic stroke is a complex process in which platelets participate either in the activated form or through the release of vasoactive substances, such as prostaglandins or serotonin. Additionally, spontaneous recanalization may produce dislodged thromboembolic material which can lead to focal hyperemia and blood-brain barrier disruption. To simulate these characteristics of human stroke, a rat model of thrombotic middle cerebral artery (MCA) occlusion-recanalization was developed. In the present study, histopathological damage was correlated with data taken from autoradiographic cerebral blood flow (CBF) studies and microcirculatory alterations in the acute phases of focal ischemia. Adult male Sprague-Dawley rats were first anesthetized with halothane. The right MCA was exposed by subtemporal approach. A platelet thrombus was induced photochemically in the proximal MCA using a He-Ne laser and the photosensitizing agent, rose bengal. Recanalization was achieved by applying nimodipine topically at 1 hr or 3 hrs following MCA occlusion. Rats were perfusion fixed on the seventh day and morphometric analysis of infarct area and volume determined. The microcirculatory state was studied by infusing carbon black at one hour after recanalization. CBF was measured by ^{14}C-iodoantipyrine autoradiography.

Permanent MCA occlusion produced consistent patterns of infarction involving neocortical and striatal areas. In contrast, histopathological analysis of recanalization groups demonstrated regional vulnerability. Small cortical infarcts, restricted to peripheral MCA territories were observed in only 2 of 6 one hr occlusion rats, whereas striatal infarction or ischemic damage was observed in all recanalized animals. Striatal infarction was confined to the dorsolateral segment. Although total infarct volume was significantly smaller in recirculated compared to nonrecirculated rats, 1 hr of MCA occlusion produced significantly larger striatal infarcts compared to 3 hrs of occlusion. Within the 1 hr occlusion group, CBF in the dorsolateral striatum at 1 hr after recanalization was relatively hyperemic compared to other parts of the striatum. Within this same experimental group, small perfusion deficits restricted to the dorsolateral striatum were observed with carbon black infusion. These results suggest that vulnerability of the dorsolateral striatum to transient focal ischemia, especially after 1 hr of ischemia, may have been caused by progressive microvascular collapse due to reperfusion injury.

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467.3 ENHANCED UPTAKE OF HUMAN β-HEXOSAMINIDASE A (HEX A) BY COUPLING TO TETANUS TOXIN FRAGMENT C (TTC) BY CNS NEURONS IN VITRO. K. Dobrenis* and M.C. Rattazzi* (SPON: J. Goldman). Dept. Pediatrics, North Shore Univ. Hospital and Cornell Univ. Med. College, Manhasset, NY 11030

Our laboratory is involved in developing rational enzyme replacement strategies for the treatment of human lysosomal storage disorders with CNS involvement. In earlier studies we found minimal uptake of Hex A by CNS neurons after transient blood-brain barrier (BBB) permeabilization in cats with GM_2 gangliosidosis. We later reported that uptake of Hex A by neural cells and neurons in culture was enhanced by carbodiimide coupling to poly-L-lysine (PLL Hex A) and non-toxic TTC (TTC Hex A), respectively (Soc. Neurosci. Abst.12:37,1986). We now have confirmed and extended the initial data, and improved the TTC Hex A conjugation by mixed disulfide formation between 2-iminothiolane-thiolated TTC and succinimidyl pyrydyl thiopropionate-derivatized Hex A. The new TTC Hex A has a higher molecular weight than Hex A by non-denaturing PAGE, retains activity with artificial substrates and GM_2 ganglioside, and forms enzymatically active immunoprecipitates with antiserum to tetanus toxin. Cellular uptake of enzyme (at 5 μg/ml) was assessed with antiserum to Hex A by immunofluorescence in embryonic day 15 rat brain mixed cell-type and neuronal cultures. Neurons were identified as tetanus toxin-binding cells, negative for glial fibrillary acidic protein and galactocerebroside.

After 1 hr, 4°C incubation with neural cultures, TTC Hex A was detectable on the surface of only neurons by both antisera to Hex A and tetanus toxin. After 18 hrs incubation at 37°C, in addition to surface binding, TTC Hex A was present in all neurons in numerous axoplasmic and karyoplasmic granules (≤1 μm dia) and larger, multivesicular-like structures indicating effective endocytosis and suggesting compartmentalization into lysosomes. After a 6 hr chase, surface bound TTC Hex A decreased, and that in karyoplasmic structures increased, suggesting continued uptake of plasma membrane-bound enzyme. In a total of eight experiments, neuronal uptake of TTC Hex A was always greater than that of PLL Hex A and much greater than that of Hex A; the latter were detectable only in small granules, primarily in small neurons. Uptake of native Hex A in the presence of thiolated TTC did not reproduce the pattern of TTC Hex A uptake, but rather that of Hex A alone.

Thus, conjugation of TTC to Hex A effectively and specifically enhances uptake of enzyme by neurons in culture. This should obviate the problem of uptake by CNS neurons after BBB permeabilization. TTC Hex A may also enhance delivery of enzyme to neurons via TTC-mediated axonal transport and transsynaptic transfer (Schwab, M.E., et al. J. Cell Biol.82:798,1979) and may allow to circumvent the BBB (Broadwell, R.D., and Brightman, M.W., J. Comp. Neurol.166:257, 1976). (Supported by NIH grant NS21404).

467.4 PERIPHERAL DEMYELINATION AND REMYELINATION INITIATED IN VIVO BY THE CALCIUM-SELECTIVE IONOPHORE IONOMYCIN. K. J. Smith and S.M. Hall*†, Department of Anatomy and Cell Biology, Eastern Virginia Medical School, P. O. Box 1980, Norfolk, VA 23501, and †Department of Anatomy, The United Medical and Dental Schools of Guy's and St. Thomas's Hospitals, Guy's Campus, London Bridge, London, SE1 9RT, England.

Incubation of nerve segments with the calcium-selective ionophores A23187 or ionomycin induces a prompt, vesicular demyelination, dependent upon a raised intracellular calcium concentration (Schlaepfer, Nature, 265:734-736, 1977; Smith, Hall and Schauf, J. Neurol. Sci., 71:19-37, 1985). We now report the events following the injection of ionomycin into the rat and mouse sciatic nerve.

Rats (Sprague-Dawley, n=64) and mice (CD1, n=42) were anesthetized, and ionomycin (6 and 0.5 μl respectively, 25 μM) was injected intraneurally using a micro-pipette. After recovery periods of 30 minutess to 2.5 months the animals were reanesthetized, perfused with glutaraldehyde (3%, pH 7.4) and the sciatic nerves prepared for light and electron microscopy. A prompt (within 30 mins.) vesicular demyelination was observed, initiated at the paranodes and Schmidt-Lanterman incisures. Segmentally demyelinated internodes were typical at 7 days. The lesions were large and, in the mouse, involved up to 95% of the fibers in the sciatic nerve. Wallerian degeneration and Schwann cell necrosis were uncommon. Not all the myelin disintegration was effected by vesiculation, for macrophage-stripping was evident in up to 30% of fibers during the first week, particularly in the rat. Macrophages also removed most of the myelin debris: myelin debris was only rarely observed in Schwann cells. Remyelination was initiated in nearly all affected fibers by 21 days, and by 2 months all the affected fibers had thin new myelin sheaths.

The appearance and spatio-temporal distribution of the myelin vesiculation was strikingly similar to that seen after phospholipase A_2, or lysophosphatidyl choline injection, consistent with a belief that an endoenous, Ca-activated phospholipase A2 may have been involved in the demyelination. The findings may be of clinical relevance with regard to demyelination mediated by the immune system.

467.5 THE SPINAL CORD PROTEIN IN BOVINE CEREBROSPINAL FLUID RISES IN EXPERIMENTAL ALLERGIC ENCEPHALITIS. C.F.C. MacPherson, J. Tupy*, L. Smith* and A. Lagace*. Dept. of Psychiatry, Univ. of Western Ontario, London, Ont. N6G 2K3, Sch. of Veterinary Med., Univ. of Guelph, Guelph, Ont. N1G 2W1 and Sch. of Veterinary Med., Univ. of Montreal, Montreal, Que. H3C 3J7.

Immunohistological experiments have shown that the anti-encephalitogenic spinal cord protein (SCP) is a component of the cytoplasm of most if not all, neurons, oligodendrocytes and Schwann cells of the bovine, human and rat nervous systems (MacPherson and Wallace, Acta Neuropathol. 50:25, 1980; MacPherson and Bonato, Acta Neuropathol. 59:191, 1983).

Recently, SCP was discovered in normal bovine and human cerebrospinal fluids. At that time it was hypothesized (1), that the SCP was secreted continuously into the CSF by neurons and oligodendrocytes and (2), that any major changes in the metabolism of neurons or oligodendrocytes would be reflected in the levels of SCP in the CSF. In order to test this hypothesis we developed a competitive inhibition enzyme immunoassay (EIA) to measure the SCP in the CSF using a rabbit anti-bovine SCP antiserum and highly purified bovine SCP (BSCP). The CSF-SCP levels were measured at varying intervals in normal bovine adults, in 6 to 8 month-old calves, and in adults in which severe experimental allergic encephalitis (EAE) was induced by injecting i.c. 250 mg whole bovine spinal cord emulsified in complete Freund's adjuvant. The daily concentration of BSCP in the CSF of normal calves did not vary by more than ±2.5 ng/mL when CSF was withdrawn about every 4 d over a month. Severe EAE was readily induced in adult cattle and clinical signs of disease were usually apparent 21 days after induction. The CSF-SCP values at the time clinical signs of EAE appeared were 2 to 3 times higher than normal. Moreover, peak SCP concentrations occurred before clinical signs were evident. These results suggest that the metabolism of neurons and/or oligodendroglia is changed in EAE and studies are continuing to ascertain which cell is contributing the excess SCP.

Supported by grants from the Research Committee, Department of Psychiatry, University of Western Ontario, the Medical Research Council and the Multiple Sclerosis Society of Canada.

467.6 T-Cell Growth Factor Activity Secreted by a New Human Glioma Cell Line. D.R.Buskirk, L.J.Marino,Jr., R.Naumann, R.B.Snow, and J.B.Zabriskie, The Rockefeller University, New York, NY 10021

As a part of our ongoing studies of the response of human T-lymphocytes to syngeneic brain tumors, cells from a human glioma were established in tissue culture. The new cell line, designated DaEs, is a rapidly growing pleiomorphic glioma cell line. Tissue culture supernatants from this line support the growth of interleukin-2 dependent T-cell lines.

Tissue culture supernatants were precipitated with 70% ammonium sulfate, dialyzed against PBS, and filtered through a 0.22 micron filter. The resulting material, concentrated ten-fold over the original supernatant, was tested for T-cell growth factor activity in both the HT-2 cell line and independent IL-2 dependent cell lines established in our laboratory. The preparation supported the growth of the cell lines at an activity of approximately 200 units/ml, where a unit is the reciprocal of the dilution which supports half maximal tritiated thymidine incorporation, making this preparation comparable in activity to our positive control, human IL-2 obtained from Boehringer-Mannheim.

The presence of T-cell growth factor activity does not necessarily imply that the cells are producing IL-2 itself. However, SDS-polyacrylamide gel electrophoresis reveals that the tissue culture supernatants contain a 15K dalton protein which migrates indistinguishably from the 15K dalton protein in the commercial IL-2 preparation.

Previous data from our laboratory demonstrated that DaEs cells respond to IL-2 by both proliferation and morphological change. Coupled with our new results, we suggest that interleukin-2 can play a broader role in tumor biology than as only a cytotoxic T-cell lymphokine.

467.7 DENDRITIC CHANGE FOLLOWING MINOR AND MODERATE TRAUMATIC BRAIN INJURY. J.T. Povlishock and D.L. Erb*. Department of Anatomy, Virginia Commonwealth University, Richmond, VA 23298.

Previous investigations of minor head injury have demonstrated that diffuse traumatically induced reactive axonal change is a consistent feature of the traumatic event, occurring without direct attendant focal change in either the related dendritic, somatic or vascular elements (J. Neuropathol. Exp. Neurol. 42:225-242; Lab. Invest. 52:540-552). As such reactive change entails the focal separation of the axon, with degeneration of the distal, detached segment, one can infer that deafferentation of the target neurons of these damaged axons underlies the morbidity associated with minor and moderate head injury. The present investigation was conducted to explore the deafferentation- induced responses triggered by such diffuse axonal damage. To this end, ultrastructural change was systematically assessed in the vestibular nuclei, wherein damaged axons are always observed following injury. Anesthesized cats were subjected to low to moderate intensity fluid- percussion injury and were followed over a ?4h to 3month survival period. Reactive axonal damage was identified through the use anterograde labeling of the cerebellar efferents with WGA-HRP, with subsequent processing for the LM and TEM visualization of the peroxidase reaction product. TEM was used to assess change in the target vestibular nuclei. Through such an approach diffuse reactive axonal change was noted throughout the vestibular nuclei, with the greatest number of reactive axons found in the inferior and lateral nuclei. Therein, in the early posttraumatic period, electron dense and detached terminals were observed in relation to numerous dendritic profiles. By the second week posttrauma, these dendrites revealed various changes including mylein figures, autophagic vacuoles and spine loss. In the second posttraumatic month, some dendrites were markedly swollen and varicose-like. These were laden with autophagic vacuoles, myelin figures and various forms of membrane debris. With survival into the third posttraumatic month, few dendritic abnormalites were seen, thereby suggesting dendritic recovery or reorganization. Collectively, all these dendritic changes occurred while many of the reactive axons were mounting a sustained sprouting response. The results of this investigation demonstrate that traumatically induced axonal damage triggers a delayed reactive change in their target neurons. The relevance of such change to the sequelae of minor and moderate traumatic brain injury will be considered.

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467.8 INHIBITION OF IN VITRO PROTEIN SYNTHESIS IN RAT CEREBRAL CORTEX FOLLOWING FLUID-PERCUSSION BRAIN INJURY. G.F. GADE* A.Alves*, P.S. Dwan*, M.K. Cheung, M.A. Verity*, and D.P. Becker Div. of Neurosurgery and Neuropathology, UCLA Medical Center, Los Angeles, CA. 90024.

While the events of brain damage in head trauma are diverse, cerebral concussion is a basic underlying phenomenon. Despite this, studies of the biochemical events in concussion are few. Such studies would seem of particular interest as, at milder levels of injury, they likely represent the fundamental response at a cellular level of the brain to mechanical trauma. The present study was undertaken in an attempt to elucidate some of these biochemical alterations. It was felt that protein synthesis would be a relatively sensitive indicator of perturbed cellular function whose impairment would have important consequences particularly in the setting of repetitive sublethal injuries requiring intact synthetic capacity for cellular recovery.

In vitro incorporation of radiolabelled amino acid was studied in a postmitochondrial supernatant (PMS) from Sprague Dawley rat cerebral cortex thirty minutes after a fluid-percussion brain insult, 1 - 2 atm in magnitude. Animals were anesthetized with an oxygen-nitrous oxide-Ethrane mixture prior to injury. Test animals recovered completely following varying periods of coma, which correlated with the severity of injury. Cell-free protein synthesis was inhibited up to 50%. Poly U-directed synthesis of polyphenylalanine was also inhibited. Sedimentation profiles of polyribosomes obtained from test animals were similar to those from controls. These findings indicate inhibition at the level of peptide elongation. They also differentiate the inhibition seen in this model from that demonstrated in cerebral ischemia with reperfusion or seizure activity. These insults result in inhibition of cell-free protein synthesis which is bypassed in the poly U-directed system, as well as accumulation of monoribosomes. Inhibition in these instances is, thus, thought to lie at the level of initiation. Therefore, the inhibition of brain protein synthesis following fluid-percussion brain injury appears unique to mechanical trauma. Continuing research aims at further defining the site and mechanism of inhibition of polypeptide elongation in this model.

467.9 CHARACTERIZATION OF ANTIGENS IN PARKISONIAN LEWY BODIES. M. Ruberg*, E. Hirsch* and Y. Agid* (SPON. A. Prochiantz). INSERM U.289, Paris 75013 France.

Monoclonal antibodies have been produced against two antigens (G7 and G9) found in Lewy bodies in dopaminergic neurons of the substantia nigra in patients with Parkinson's disease. The antigens have been characterized by immunoblot after two dimensional gel electrophoresis. The molecular weights and isoelectric points are: 40kd and 5.6 for G7; 70kd and 7-8 for G9. G7 and G9 are found in the substantia nigra and locus coeruleus, G9 is found as well in the striatum, cortex and cerebellum terminal regions of the nigrostriatal and coeruleocortical neurons. G7 is not found in the essentially cholinergic substantia innominata, although Lewy bodies form in this structure. The antigens may be markers of catecholaminergic neurons and, in the case of G9, nerve terminals. Present in the substantia nigra of a newborn infant before neuromelanin formation, they are not related to the presence of pigment in these cells. The antigens do not comigrate with, and are not labelled by antibodies against other substances (neurofilaments and tyrosine hydroxylase) previously identified in Lewy bodies, or a variety of cytoskeletal proteins. They are found in normal subjects and in patients with progressive supranuclear palsy, a parkinsonian syndrome without Lewy bodies, and do not therefore cause Lewy body formation. Absent from the substantia innominata, they are not structurally essential for Lewy body formation. The identity of the antigens remains unknown. It is hoped that it will be determined by sequencing after cloning of the corresponding cDNA. The localization of the antigens in normal neurons and in Lewy bodies is being studied by electron microscopy, as a first step in the analysis of the process that leads to their incorporation into the corpuscle.

467.10 RESTRICTION FRAGMENT LENGTH POLYMORPHISM STUDY OF CANINE NARCOLEPSY. M. Motoyama*, T.S. Kilduff, W.C. Dement, B.S.M. Lee*, H.O. McDevitt* (SPON: K.I Kaitin). Depts. of Psychiatry and Medical Microbiology, Stanford University School of Medicine, Stanford, CA 94305.

Narcolepsy, a disorder characterized by periods of excessive daytime sleepiness and cataplexy, has been reported in humans and dogs. In humans, the disease is believed to be inherited by a polygenic mechanism, while in Labrador retrievers and Doberman pinschers, an autosomal recessive type of inheritance is implicated. In humans, a high association between one class II allele (HLA-DR2) and narcolepsy has been shown by Honda et al. We are performing studies to establish whether such an association exists in narcoleptic Labrador and Doberman families and whether homozygosity within the major histocompatibility complex (MHC) is necessary for expression of the disease.

It has been established that restriction fragment length polymorphism (RFLPs) reflect serologically and cellularly defined alleles of the class II region of the MHC. With the use of two locus-specific human class II cDNA probes (HLA-DQβ and HLA-DRβ) and several restriction enzymes (Bgl II, Pst I, Hind III, Eco RV, Bam HI), we analyzed several dog families for genetic polymorphism in the MHC region in which at least one parent was narcoleptic.

Preliminary data suggest that the genetic pattern for narcolepsy is not consistent with homozygous expression in the MHC loci. Restriction patterns in genomic DNA cut with Pst I and probed with the DRβ gene show that Doberman and Labrador families do not share the same haplotype in the MHC region.

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467.11 CANINE DLA TYPE AND NARCOLEPSY: IS THERE AN ASSOCIATION: R. Dean,* T.S. Kilduff, W.C. Dement and C. Grumet* (SPON: M. Ellis) Depts. of Psychiatry and Pathology, Stanford University School of Medicine, CA. 94305

Narcolepsy is a neurological disorder characterized by excessive daytime sleepiness, sleep onset REM periods, and cataplexy. Population studies have demonstrated that almost 100% of patients possess the major histocompatibility complex (MHC) Class II antigen, HLA-DR2 compared to only 22-34% for controls. These findings along with family studies are most consistent with mulitgenic control of this disease. These data suggest that with the "proper" background, linkage disequilibrium with a specific ClAss II allele or allele complex is a strong contributing factor in narcolepsy susceptibility.

The canine narcolepsy model resembles the human disease both behaviorally and pharmacologically. In the selected genetic background provided by several breeds of dogs (Doberman pinscher and Labrador retriever), the disorder appears to be transmitted as a single-gene, autosomal, recessive trait, canarc-1. Analyses of one-way mixed lymphocyte culture patterns of reactivity among narcoleptic dogs reveals extensive heterogeneity with minimal sharing of Class II alleles. The canine data indicate: (1) that rather than a single Class II allele association (as found in humans), many different Class II alleles are found in the dog model of narcolepsy; (2) for some specific narcolepsy genes, such as canarc-1, linkage disequilibrium to a MHC allele may be an epiphenomenon or not be a necessary element for the manifestation of disease. The disease in humans appears to be multigenic. If the preliminary findings of non-association between the canarc-1 gene and a specific MHC allele holds, then the dog model may provide a means of identifying the corresponding non-MHC gene(s) involved in the human disorder.

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467.12 A MOTOR NEURONAL CELL SURFACE ALTERATION IN ALS. S. Wright*, E.K. Bjornskov*, D.T. Stephenson* and P.D. Kushner. ALS Research Ctr., Pacific Presbyterian Medical Ctr., San Francisco, CA 94115.

ALS is a neurodegenerative disorder of the motor neuron system. We are examining ALS nervous tissue to describe the molecular pathology occurring in the course of the disease. Tor 23 is a monoclonal antibody (JNC 43 775) which binds the surface of motor neurons in Torpedo (PNAS 80 7342), rodents (see abstract, Stephenson et al., this volume) and humans (Muscle & Nerve, in press), thus demonstrating a potentially important epitope in motor nerve structure and function. This study focuses on whether, with Tor 23, we can observe consistent changes occurring along the surface of motor neurons in ALS, from their central origins to their peripheral terminations.

Tor 23 was localized with immunofluorescence on frozen sections cut from human intercostal muscle and ventral root. In normal tissue, Tor 23 binds the external perimeter of the Schwann cell and the axon. Thus, in a cross-section, one sees an outer and inner ring of stain, or a donut configuration. The closely packed nerve bundles display a uniform deposition of donut stain around all axons, with no stain in the interstitial space. In the ALS tissue, 1) there is a differential deposition of stain in the donut structure: on some fibers there is a heavy asymmetric concentration of punctate stain around the exterior sheath in a distinct semi-lunar configuration, 2) the broad interstitial space which is present in ALS nerve bundles is diffusely stained, 3) there are distinct holes of no stain, which may be a loss of individual axons or a loss of immunoreactive material on degenerating axons. These alterations were consistently observed in both peripheral nerve bundles and ventral roots. An examination of the ventral root in longitudinal aspect from normal tissue reveals Tor 23 binding all along the apparent axonal surface with a uniform intensity of stain in the region of the node of Ranvier. In ALS, a greater and a non uniform deposition of stain occurs in the node region. This alteration may describe an incipient demyelinating process, but why this region should accumulate antigenic material is not clear.

We have colocalized Tor 23 with anti-neurofilament antibody, as an internal marker for the axon. Nerve bundles from normal cases show a 1:1 correspondence of neurofilament and Tor 23 stain. In ALS, the 1:1 ratio is disturbed in that neurofilament is present but Tor 23 staining is faint or absent. The opposite, neurofilament absent with Tor 23 present, is also observed. This change in staining pattern may represent a selective loss of a surface molecule in degenerating fibers and an accumulation of that molecule in regenerating fibers. Of course, the inverse may also be true. The power of this monoclonal antibody is its potential to home in on the molecule undergoing change.

467.13 LOCALIZATION OF 70 KDa HEAT SHOCK PROTEIN INDUCTION IN GERBIL BRAIN AFTER TRANSIENT ISCHEMIA. T. S. Nowak, Jr.*, K. Vass* and W. J. Welch*† (SPON: G. Dienel). Laboratory of Neuropathology and Neuroanatomical Sciences, NINCDS, NIH, Bethesda, MD 20892 and †Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

The distribution of stress protein induction in gerbil brain after transient ischemia was examined using a mouse monoclonal antibody which recognizes inducible forms of 70 kDa heat shock protein (hsp70). Gerbils were anesthetized with 2 % halothane in 70% N_2O/30% O_2 and subjected to 10 min bilateral carotid artery occlusion. After recirculation intervals of 4-48 hr brains were fixed by transcardial perfusion with 4% paraformaldehyde in phosphate buffer and processed for immunocytochemistry using peroxidase detection systems. Control brain showed immunologically detectable material localized exclusively to ependymal cells lining the ventricles. Following ischemia additional reactivity appeared in various brain regions with a characteristic time course. After 4 hr moderate staining was detectable in hippocampal dentate granule cells, amygdala, and the medial nucleus of the habenula. By 12 hr, labeling of these cells intensified, and there was evident staining of large striatal nerve cells and of a number of basal forebrain and cortical regions. Dentate granule cells remained positive at 24 hr, CA3 began to show reactivity, and CA1 showed occasional weak staining. Positive staining was evident in numerous cortical neurons at this time. At 48 hr CA3 was even more intensely positive, while there appeared to be diminished reactivity in dentate; no reactivity was evident in CA1, and cortical labeling was also reduced. Throughout these studies hsp70 reactivity was found only in the cytoplasm and occasionally the nucleolus of neurons and ependymal cells. Glial cells, vascular elements and meninges were negative. Whether ependymal staining in control animals accounts for the low level of hsp70 detectable in control brain, or represents another cross-reacting protein remains to be determined. The time course of increases in hsp70 immunoreactivity agree well with that observed for whole brain hsp70 mRNA levels and hsp70 synthesis rate, both of which peak at approximately 12 hr recirculation and remain above control at 24 hr. With regard to localization, it is of interest that many positive cells are found in hippocampus and other limbic structures, circuitry which may be the anatomical substrate for proposed excitotoxic mechanisms of neuronal loss after ischemia. Strikingly, accumulation of hsp70 was apparent in CA3, dentate granule cells and large striatal neurons which survive transient ischemic insults, but was greatly attenuated in CA1 and small striatal neurons which do not. This heterogeneity in hsp70 induction may reflect variations in post-ischemic metabolic stress as well as in the response to stress of various cell populations.

467.14 CUMULATIVE BRAIN INJURY IN REPEATED ISCHEMIC INSULTS. S. Xu*, H. Wagner, S. Tomida*, K. Vass*, T. S. Nowak Jr.*, R. Pluta*, I. Klatzo. LNNS, NINCDS, NIH, Bethesda, MD 20892.

The effect of repeated ischemic insults on a number of physiological parameters was evaluated in a gerbil model, in which an implanted device allowed repetitive bilateral common carotid artery occlusion of any desired duration and frequency. In particular, we have compared the consequences of three 5 min occlusions with those resulting from single 5 and 15 min occlusions. All occlusions were done under anesthesia with 2% halothane in 70% N_2O, 30% O_2. Measurements in brain included: regional cerebral blood flow, determined by [^{3}H] nicotine uptake; regional water content determined as specific gravity; regional vascular volume as the distribution space of [51Cr]-labeled red blood cells; and tissue oxygen tension determined polarographically with platinized microelectrodes. The status of brain microcirculation was also evaluated morphometrically by the counting of vascular channels filled with Evans blue under fluorescence microscopy. Cortical blood flow fell from control values of approximately 200 ml/100 gm/min to undetectable levels during occlusion, and returned briefly to the control range at 2-3 min. Recirculation was followed within 15 min by the characteristic period of hypoperfusion with flow averaging 30-35 ml/100 gm/min. A similar pattern was seen in hippocampus and caudate, while blood flow in cerebellum did not change. Following repeated occlusion, peak values at 3 min recirculation were only 60% of control, but hypoperfusion was identical after each occlusion. Significant recovery occurred within 6 hrs recirculation following either single or multiple occlusions, and no reduction in flow persisted at 24 hrs. Moderate edema followed single occlusions, tending to recover by 6 hrs recirculation. Edema increased progressively with repeated insults, but also tended toward recovery at 6 hr. At 24 hrs following repeated occlusions, however, a second phase of marked edema was noted in all brain regions subjected to ischemia, with the cumulative effect significantly greater than that observed following a single 15 min occlusion. Total vascular volume was reduced by 40-50% during the first 6 hrs following repeated occlusions, tending to recover in cortex by 24 hrs but remaining low in thalamus, striatum and hippocampus. Capillary filling was reduced in proportion to brain edema. Tissue oxygen tensions did not recover promptly after successive cycles of occlusion and recirculation, consistent with diminished capillary perfusion. These results suggest that progressive secondary hypoxia may play a role in the dramatically increased edema and tissue injury following repeated ischemic episodes in gerbil brain.

467.15 SMALL DIFFERENCES IN INTRA-ISCHEMIC BRAIN TEMPERATURE CRITICALLY DETERMINE THE EXTENT OF ISCHEMIC NEURONAL INJURY. R. Busto, M.Y.-T. Globus, W.D. Dietrich, P. Scheinberg, and M.D. Ginsberg, Cerebral Vascular Disease Research Center, Department of Neurology, University of Miami School of Medicine, Miami, FL 33101.

We have tested whether small intra-ischemic variations in brain temperature influence the outcome of transient ischemia. To measure brain temperature, a thermocouple probe was placed stereotaxically into the left dorsolateral striatum of rats prior to 20 min of 4-vessel occlusion. Rectal temperature was maintained between 36-37°C by heating lamp, and striatal temperature prior to ischemia was 36-37°C in all animals. Six animal subgroups were investigated, including rats whose intra-ischemic striatal brain temperature was not regulated, or was maintained at 33°C, 34°C, 36°C, or 39°C. Postischemic brain temperature was regulated between 36-37°C except for one group in which brain temperature was lowered from 36°C to 33°C during the first hour of recirculation. Energy metabolites were measured at the end of the 20 min ischemic insult, and histopathological evaluation carried out at 3 days postischemia.

Intra-ischemic variations in brain temperature had no significant influence on energy metabolite levels measured at the conclusion of ischemia. The histopathological consequences of ischemia, however, were markedly influenced by variations in intra-ischemic brain temperature. In the hippocampus, CA1 neurons were consistenly damaged at 36° but not at 34°. Within the dorsolateral striatum, ischemic cell damage was present in 100% of the hemispheres at 36°C but only in 50% at 34°C. Ischemic neurons within the central zone of striatum were not observed in any rats at 34°C but in all rats at 36°C. In rats whose striatal temperature was not controlled, brain temperature fell from 36°C to 30-31°C during the ischemic insult. In this group, no ischemic cell change was seen within striatal areas and was only inconsistently documented within the CA1 hippocampal region. These results demonstrate that despite severe depletion of the brain energy metabolites during ischemia at all temperatures, small increments of intra-ischemic brain temperature markedly accentuates histopathological changes following 3-day survival. These finding demonstrate that brain temperature must be controlled above 33°C in order to ensure a consistent histopathological outcome. Preliminary findings have demonstrated that decreases in intra-ischemic brain temperature also attenuate the release of striatal glutamate and dopamine. Correlations between neurotransmitter release and ischemic neuropathologic findings are currently under investigation. Supported by USPHS Grant NS 05820.

467.16 SODIUM AND CALCIUM ION INFLUX CONTRIBUTES TO ULTRASTRUCTURAL DAMAGE IN CULTURED NEURONS AFTER NEURITE TRANSECTION. D. G. Emery, J. H. Lucas, and G. W. Gross. Dept. of Zoology, Iowa State University, Ames, IA 50011 and Dept. of Biological Science, North Texas State University, Denton, TX 76203.

Ultrastructural changes in cultured spinal neurons after dendrite transection include swelling of the cisternae of Golgi/smooth endoplasmic reticulum (G/SER), swelling of mitochondria, and disappearance of microtubules (Emery et al., Ex. Brain Res., In Press). Changes begin near the lesion and spread toward the soma, producing a gradient of damage in the transected dendrite. To test the hypothesis that these changes are due to influx of calcium ions we transected dendrites 100 µm from the soma with a U-V laser in calcium-free medium (Gibco), or in medium with choline chloride replacing sodium chloride, or in choline-substituted calcium-free medium. Cultures were rinsed twice with experimental medium before the cell surgery. Cultures were fixed at intervals to 20 minutes after cell surgery and examined by transmission electron microscopy. We examined eleven cells lesioned in calcium-free medium, eleven with sodium-free medium, thirteen in medium lacking both sodium and calcium, and four in calcium-free medium to which EGTA had been added.

In all experimental media mitochondria showed few changes except very near the lesion, and microtubules did not degenerate except very near the lesion. G/SER cisternae began to swell in the injured dendrite, and this spread toward the soma. Swelling of the G/SER proceeded more slowly than in normal medium and appeared sooner in low-calcium medium than in sodium-free medium. Changes occurred most slowly in low-calcium, sodium-substituted medium. The fewest changes were seen in low-calcium medium with EGTA. These results indicate that there may be residual calcium ions after two rinses with calcium-free medium (analysis by atomic absorption spectrophotometry showed 30µM or less calcium). EGTA may have bound the residual free calcium, indicating that calcium influx is a major cause of organelle damage after injury. Sodium influx also appears to be involved, perhaps by liberating calcium ions from intracellular stores. The apparent synergism of calcium and sodium influxes may be due to the additive effects of residual free calcium in the medium and sodium mediated intracellular calcium release.

467.17 VERAPAMIL DOES NOT ENHANCE SURVIVAL OF CULTURED MAMMALIAN SPINAL NEURONS AFTER NEURITE TRANSECTION. R.Y.Shi*, J.H.Lucas, and G.W. Gross. (SPON: P. GUTHRIE) Dept. of Biological Sciences, P.O. Box 5218, North Texas State University, Denton, TX 76203.

The ultrastructural deterioration and cell death following a variety of lethal insults including hypoxia and physical trauma have been correlated with an increase in the intracellular concentration of free calcium. Calcium channel blockers have been demonstrated to be effective for reduction of renal cell death after anoxia and for minimization of the area of necrosis after myocardial infarction. The purpose of these studies is to determine the efficacy of calcium antagonists to protect nerve cells after a physical injury of known severity.

Spinal cords from 13-14 day mouse embryos were dissociated and grown in monolayer cultures for 3-4 weeks. Matched pair experiments were performed using two cultures from the same seeding date. In each culture a primary dendrite was transected from each of ten experimental cells at a lesion distance of 100 µm from the perikaryon. Ten other neurons served as unoperated control cells. Cell surgery was performed using a UV laser microbeam as previously described (Gross et al., J.Neurosci. 3:1979, 1983; Lucas et al., CNS Trauma 2:231, 1985). The medium of one culture was mixed with 10 µl/ml of 8×10^{-3} M verapamil (8×10^{-5} M final conc.) 2 h prior to laser cell surgery. The control culture received an equal volume of saline 2 h prior to surgery. Unoperated spinal neurons show 100% viability after a 4 h exposure to verapamil at this concentration. We find that a four hour exposure to verapamil is toxic (less than 100% viability) to spinal neurons in culture starting at a concentration of 10^{-4} M.

Control and operated cell viability was evaluated 2 h after surgery using phase contrast (PC) observation and at 24 h using a combination of PC observation and erythrosine B dye exclusion. After the 2 hr observation all of the medium from each culture was removed and replaced with fresh conditioned medium (no verapamil).

We have performed 14 experiments (7 pairs). At 2 h after lesioning the mean operated cell survival in the presence of verapamil was 51±18%. Two hour survival of operated cells in medium without verapamil was 55±17%. Mean survival of the two groups at 24 h was 38±16% and 51±14% respectively. Unoperated cell survival in each group was 100% at 2 h and 24 h.

These preliminary data indicate that verapamil fails to protect neurons after physical injury and may even increase the likelihood of death among lesioned cells. This finding tends to support earlier studies which have suggested that events occuring at the lesion rather than general changes in ion channel conductance determine the fate of injured nerve cells. Further studies with verapamil as well as other calcium antagonists and channel blockers will be conducted. Supported by PHS grant 2 RO1 NS23686-03.

467.18 SURVIVAL OF CULTURED MAMMALIAN SPINAL NEURONS AFTER NEURITE TRANSECTION INJURY IS ENHANCED BY PROXIMAL SEGMENT RETRACTION. J.H.Lucas, L.E.Czisny* and G.W.Gross, Dept. of Biological Sciences, P.O. Box 5218, North Texas State University, Denton, TX 76203.

We have shown that nerve cell survival after amputation of primary dendrites within 250µm of neuronal perikarya is a function of the physical parameters of the transection lesions (Lucas, J.H. et al., CNS Trauma 2:231, 1985). The probability of survival increases with increasing lesion distance and is inversely proportional to lesion diameter. We now wish to report that retraction of the proximal segment of a severed neurite constitutes a third lesion physical parameter which enhances the probability of nerve cell survival after transection injury.

Dissociated mouse spinal cords from 13-14 day embryos were grown in monolayer cultures for 3-4 weeks. Primary dendrites were transected from 10 neurons in a culture while 10 neurons in the same culture served as unoperated control cells. Cell surgery was performed as previously described using a UV laser microbeam (ibid.; Gross, G.W. et al., J.Neurosci. 3:1979, 1983). A primary neurite was amputated at a lesion distance of 100µm from the edge of the perikaryon of each experimental cell. After lesioning, a single laser shot was used to make a small crack below the surface of the culture substrate at the target point. This mark served as a visible reference for subsequent observations of retraction. Measurements of lesion diameter and retraction were made from photographs taken just before and 5 min after surgery.

Data from over 150 observations were separated into 3 groups by lesion diameter (1.5-2.4µm, 2.5-3.4µm, 3.5-4.4µm). Within each group the data were further organized based upon the extent of proximal segment retraction from the transection point (0-5µm, 6-10µm, etc.). The percent viability of the cells in each retraction bin showed clearly that, as the extent of retraction increased, the likelihood of nerve cell survival also increased in a linear fashion ($r > 0.84$ in all cases). For example, within the 2.5-3.4µm diameter group the probability of survival increased from 33% when retraction was 0-5µm to 56% when retraction was 21-25µm. We also calculated the retraction/lesion diameter ratio (R/D) for each operated cell and the probability of survival for cells at each R/D value. The resulting function demonstrated that the probability of nerve cell survival increased linearly with increasing R/D ($r=0.94$).

Enhancement of the probability of nerve cell survival by retraction of the proximal segment is most likely the result of increased resistance to injury currents (Ca^{+2} and Na^{+}) penetrating the cell at the lesion. This increased resistance is probably due to more efficient membrane resealing as well as to accumulation of organelles and subcellular structures at the tip of the severed neurite ("debris damming"). These data again indicate that events at the lesion determine the probability of neuron survival after physical injury close to the perikaryon. (PHS NS23686-03)

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY VI

468.1 EMBRYONIC DEVELOPMENT OF THE RETINOTECTAL PROJECTION IN ZEBRAFISH. C.A.O. Stuermer and J. Vielmetter*. Friedrich-Miescher-Lab., Max-Planck-Institute, D-7400 Tuebingen, FRG.

For ongoing investigations on axonal navigation in the visual system of zebrafish, a timetable of axonal growth in the developing retinotectal pathway had to be established.

Zebrafish embryos were staged (10 Min) within 1 h post fertilization (PF) and incubated at 28°C. HRP was applied to the developing retina at hourly intervals between 30 - 52 h PF and at 55, 70, 72 h, 4 and 9 d PF. After fixation, the HRP-labeled axons were visualized in DAB-reacted vibratome sections (30 - 46 h) and in whole mounts of the brain (40 h ~ 9 d).

The first retinal axons, typically 1 - 5 pioneers with simple elongated growth cones, smaller than 5 µm in length enter the optic stalk between 34 and 35 h PF. As the pioneers approach the chiasm (60 µm from eye) a second group of 5 - 10 axons appears in the stalk running parallel to, or closely attached to the pioneers at a distance of 25 - 45 µm. When the pioneers turn dorsally at the periphery of the diencephalic wall close to the contralateral eye, a front of numerous axons has entered the stalk.

On their path towards tectum the pioneers are led by simple or lamellopodial growth cones. Filopodial branches emerge from some axons over 100 µm proximal to the growth cone. The second group and the front of axons follow the pioneers at distances of c. 50 to 100 µm.

The time at which axons reach tectum varies among fish. In some, pioneers arrive at tectum at 46 h PF, having covered a distance of 330 µm (uncorr. for shrinkage) in 12 h (mean velocity: 27.5 µm/h), in others up to 4 h later.

The pioneers invade from separate points rostrally into tectum. Their leading tip begins to broaden and to emit short collaterals. The tectum itself consists of 2 domains, a central core (180 x 100 µm) to which the axons are restricted and a half moon shaped zone of elongated undifferentiated cells surrounding the core region medially, laterally and caudally. Between 52 and 55 h many more axons have invaded into the rostral half of the core region, with growth cones closely apposed to the most advanced axons. The latter often carry several branches of c. 10 µm length arising over 50 µm of the axon's distal end. Occasionally one of the branches is tipped with a growth cone. By 70 h, axons have filled the entire core region (now 250 x 190 µm) and small terminal arbors of 35 x 45 µm are discernible. From 70 - 72 h onwards, after HRP-applications localized to retinal quadrants, labeled axons were found in retinotopically corresponding tectal quadrants. The formation of a retinotopic map coincides with the time at which zebrafish embryos hatch.

468.2 **FORMATION OF THE RETINOTECTAL PROJECTION IN LARVAL GOLDFISH.** **P. A. Raymond and C. A. O. Stuermer. Univ. Michigan, Dept. Anat. & Cell Biol., Ann Arbor, MI and Friedrich Miescher Lab., Tuebingen, West Germany.**

The organization and regeneration of the retinotectal projection has been thoroughly investigated in adult goldfish, but nothing is known about its development. We used anterograde transport of HRP and retrograde transport of Fluoro-Gold to label optic fibers in larval goldfish from hatching to 2 wks old.

Two ages of larval goldfish were used: newly hatched to 2 days old and 2 wks old. For HRP labeling, a small crystal of HRP was inserted into one eye. Optimum transport times were 15 mins (2-day-old fish) and 3 hrs (2-wk-old fish). Tecta were reacted with DAB then whole-mounted or embedded in methacrylate and sectioned. For Fluoro-Gold labeling, 20 nl of a 4% solution was injected intracranially over the right tectum (2 wk old fish only). Two to 4 wks later, eyes were whole-mounted and brains were cryosectioned and examined with fluorescence.

In the youngest larvae, fibers projected to tectum; most were tipped with growth cones and some with terminal arbors (median size of deep arbors: 35 x 55 um). Median arbor area as a fraction of tectal area was 2.7%. In older larvae, arbors were larger (median: 50 x 100 um), but the relative coverage was the same (median: 3.2% of tectal area). In adults, terminal arbors in deep SFGS (F_4) are 100 x 150 um (Stuermer, 1984, J.Comp.Neur. 229:214), but they each occupy only a small fraction (0.15%) of tectal area. The shapes of terminal arbors in larval tectum resembled those in adults (Y-shaped primary bifurcation with higher-order branching). We tried to apply HRP to a localized region of retina, and in some older larvae, all labeled terminals clustered in a patch that covered less than 10% of tectal area. This observation provides preliminary evidence for retinotopic order. Labeled axons in nerve and tract were fasciculated. Ganglion cells throughout the retina were retrogradely labeled by diffuse Fluoro-Gold injections. We conclude that optic fibers have reached the tectum by the time of hatching in goldfish and that formation of terminal arbors takes place during larval development. When the first synapses are made is not yet known. **(Supported by PHS EY04318, the Alfred P. Sloan Foundation (PAR) & Friedrich Miescher Laboratorium (CAOS).**

468.3 THE ESTABLISHMENT OF TOPOGRAPHICAL ORDER IN MAMMALIAN RETINOTECTAL PROJECTIONS: INSIGHTS FROM TRANSPLANTATION STUDIES.
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Mammalian retinotectal projections show a remarkable degree of topographical order from the time they are first established. Little is known of the mechanisms which determine this order. Transplantation techniques provide a novel insight into such mechanisms.

Embryonic rat retinae transplanted to neonatal brain differentiate and develop normally. All the retinal cell types are present and organized in layers as in the normal retina. Fibers arising from the ganglion cells of the transplant follow pathways different from normal but project only to normally retino-recipient structures in the host brain.

We have studied the topographical order of these projections. Retinae from 14 day-old rat embryos were transplanted either on the surface or deep into the midbrain of neonatal rats. The right or both the host eyes were removed on the same day. One to 8 months later the animals were anesthetized, the left superior colliculus (SC) was exposed and two small non-overlapping injections, one of Fluorogold and one of rhodamine beads, were made in the superficial layers of the SC.

In normal rats similar non-overlapping injections resulted in two spatially separated regions of labelled cells in the retina, segregated by label. In transplanted animals, cells labelled with either dye were found scattered throughout the ganglion cell layer of the transplant. Moreover, in all cases cells labelled with one dye were found interspersed among cells labelled with the second dye, with a complete lack of segregation by label. Control studies show that this scatter of labelled cells is not due to uptake of label by fibers of passage.

Therefore, we conclude that transplanted retinae do not project in a topographical order to the host SC.

These results suggest that near-neighbourness of retinal ganglion cells is not a sufficient condition for the proximity of their terminal fields. Supported by NIH grant EY05283.

468.4 **RETINAL TRANSPLANTS FAIL TO SHOW TARGET-DIRECTED OUTGROWTH IN ANOPTHALMIC MICE.**
M.H. Hankin and R.D. Lund. Department of Neurobiology, Anatomy and Cell Science, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261

We have found that retinal transplants placed deep in the midbrain show a directed outgrowth through the brain parenchyma to the superior colliculus (*Brain Res.* **408**: 344 (1987)). This suggested that cells in the colliculus exert a tropic, or trophic, influence on transplant-derived axons. The present studies address the question of whether a superior colliculus that had not *previously* received optic input during normal development could still attract retinal axons.

Embryonic (E13) Sprague-Dawley rat retinae were transplanted into the cerebral aqueduct of normal newborn mice (CD-1) or to a strain caontaining the recessive gene which results in ocular retardation (or^J). In the heterozygous condition ($or^J/+$) the animals appear normal, whereas the homozygous form (or^J/or^J) is anopthalmic. After 3 weeks the animals were perfused with 4% paraformaldehyde and their brains cryoprotected with 30% sucrose and sectioned on a freezing microtome at 30 µm. Transplant projections were examined with a normal silver stain or with a degeneration stain in cases where immune rejection of the graft was precipitated by a skin graft placed on the flank of the animal.

Retinae placed in mice in which one eye was removed at birth (CD-1 or $or^J/+$) exhibited a vigorous and directed outgrowth towards the denervated superior colliculus. This projection was distributed throughout the deafferented superficial layers of that colliculus. In contrast, little or no outgrowth could be detected from retinae placed in littermate mutant animals (or^J/or^J), although ganglion cells could be identified in these retinae. In the occasional case where limited outgrowth was seen in the mutant animals, the large fasciculi characteristic of the projection into normal animals were not observed and the fibers failed to show a strong orientation towards the superior colliculus.

Although it is possible that the or^J genetic defect may directly affect the superior colliculus itself, the present observations suggest that in order for the colliculus to exert a positive influence on optic axons (either trophic or tropic in nature) it must have received prior innervation. This raises the possibility that the first optic axons to reach the tectum may induce the production of a target-derived factor which affects the ingrowth of subsequent axons.

Supported by NIH grants NS07817 (MHH), EY05283 and EY05308 (RDL).

468.5 **DECUSSATION OF RETINAL GANGLION CELL PROJECTIONS DURING PRENATAL DEVELOPMENT OF THE CAT.**
B. Lia, M.A. Kirby[1], and L.M. Chalupa, Dept. of Psychology, Univ. of California, Davis, CA 95616, [1]Depts. of Pediatrics & Anatomy, Sch. of Med., Loma Linda Univ., Loma Linda, CA 92350.

In the adult cat, the pattern of decussation of retinal ganglion cell projections demarcates the nasal from the temporal hemiretina. The prenatal development of this nasotemporal divison was studied in fetal cats of known gestational age. Following successful injections of tracers (HRP, rhodamine latex microspheres, diamidino yellow, fast blue) into one optic tract or injections of a different tracer into each tract, the distribution and number of retrogradely labeled ganglion cells was determined in retinal wholemounts.

At all ages examined, from the period of maximum ganglion cell number--day 38 of the cat's 65 day gestation--until birth, a pronounced nasotemporal division was evident. Similar to the adult pattern, the ipsilateral projection had a sharper decussation boundary than the contralateral projection, the latter extending across the decussation line into temporal retina. However, refinement of the decussation pattern was observed during prenatal development. With advancing fetal age, there was a progressive decrease in both the proportion of the uncrossed population found in nasal retina and the proportion of the crossed population found in temporal retina. Particularly apparent for the uncrossed nasal population, as reported for the neonatal cat (Jacobs et al., 1984), 'inappropriately' projecting cells were more numerous in the retinal periphery. Interestingly, in related studies we have found that the few cells of the retinoretinal projection present during fetal development also tend to be found in the peripheral retina.

Double-labeled ganglion cells were very rarely encountered following injections of a different fluorescent tracer into each optic tract. Thus refinement of the decussation pattern is due to ganglion cell loss rather than collateral elimination. However, the degree of ganglion cell loss underlying the correction of fetal retinal decussation patterns is minor relative to the massive overproduction and elimination of ganglion cells during normal development.

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468.6 A NEONATAL CROSSED ABERRANT, EXUBERANT PROJECTION TO THE INFERIOR COLLICULUS, VISUALIZED IN WHOLE-BRAIN PREPARATIONS OF THE PIGMENTED RAT.
A.M. Cooper* and A.Cowey* (SPON:M.C.Brown) Dept. of Expt. Psychology, Oxford, OX1 3UD, England.

Unilateral eye injections of Horseradish Peroxidase (HRP) were given to Long-Evans pigmented rats, either when adult or on postnatal day 0, 1, 2, 3, 4, 5 or 10. 'Whole-brains' were prepared for HRP-histochemistry by removing the cortices and hippocampus, thus exposing the thalamus and midbrain. A new method for the histochemical reaction of HRP in such 'whole-brains' was developed, using Tetramethylbenzidine (TMB) as the chromogen and Ammonium Heptamolybdate (AHM) as a stabilizing agent. This method has previously been used only on sectioned material. HRP-labelled optic pathways could thus be visualised, during development, across the thalamus and midbrain. After being photographed the preparations may be sectioned and then re-reacted and/or counterstained, enabling a 3-D reconstruction of the optic pathways to be made. There is evident at birth a crossed retinal projection to the inferior colliculus (IC). The labelled fibres enter the IC medially at its anterior border with the superior colliculus (SC) and course across the IC, curving and spreading out in a caudo-lateral direction. The fibres arch over the caudal pole of the IC ventrolaterally and turn rostrally into the IC. From light microscopy of the sectioned material it appears that the fibres terminate in the pericentral nucleus of the IC. The projection attains its peak density between postnatal days 1 and 2 after which it retracts towards the rostral IC. By postnatal day 5 it covers only a small area at the rostral border between the IC and the SC, and is totally eliminated by postnatal day 10 and in the adult Long-Evans rat. There is no evidence of an uncrossed retinal projection to the IC at any age in these pigmented rats. Injections of fluorescent tracers into the IC on postnatal day 1 show that this neonatal transient projection, which spans two sensory systems, arises from ganglion cells scattered across the entire contralateral retina. There is a suggestion of at least crude topographical organization from the retina to the IC which merits further research. No labelled retinal ganglion cells are present after similar injections into the IC of adults.

468.7 THE ROLE OF THE NMDA RECEPTOR IN EYE-SPECIFIC SEGREGATION IN THREE EYED TADPOLES. H.T. Cline & M. Constantine-Paton. Biology Dept. Yale University, New Haven, CT. 06511.

We are using the three-eyed frog to examine the development of segregation of retinal afferent terminals into eye-specific stripes. APV, a specific NMDA receptor antagonist causes stripe desegregation when applied chronically to the tectum [Cline et al, ('87) PNAS 84(12)]. The NMDA receptor/channel conducts calcium only when the receptor binds transmitter while the membrane is simultaneously depolarized by other inputs. We suggest that activation of the NMDA receptor allows the tectal neurons to recognize and maintain coactive afferent synapses.

Application of NMDA to the tecta of three-eyed tadpoles has the curious effect of producing straighter stripes with sharper borders compared to untreated animals. We counted the number of HRP-labeled axons from the third eye which cross from stripe to interstripe zones. Regions of tecta exposed to NMDA have less than half the number of HRP-labeled processes crossing from stripe to interstripe zones compared to untreated regions of tecta. Exogenous NMDA may desensitize the NMDA receptors so that a higher level of afferent coactivity is required to trigger synapse stabilization, resulting in sharper borders.

To pursue the response to NMDA and APV, we are developing a tectal slice preparation. Slices are cut on a vibratome and visualized on a fixed stage microscope equipped with a cooled substage. Intracellular recordings indicate that the neurons are healthy, with resting potentials in the range of -50 to -70 mV. Although some neurons are relatively silent, many exhibit continuous electrical activity and can be driven to fire action potentials repeatedly with anode break excitation. We have labeled small populations of neighboring retinal ganglion cells with TRITC. Slices can be cut through the tectum and the diencephalon to include a few dye-filled axons in the optic tract and their terminal fields in the tectum. This preparation allows electrical stimulation of the retinal inputs while recording from their target tectal neurons. We plan to characterize the tectal neurons according to electrophysiological and pharmacological criteria. This work was supported by NIH grants EY05818 & EY06039.

468.8 KYNURENIC ACID BLOCKS RETINAL-TECTAL TRANSMISSION IN *RANA PIPIENS*. E. A. Debski, H. T. Cline and M. Constantine-Paton. Dept. Biology, Yale University, New Haven, CT 06511.

It has recently been shown (Cline et al., PNAS, 84 (12), 1987) that the *N*-methyl-D-aspartate (NMDA) receptor antagonist amino-phosphonovaleric acid (APV) prevents the eye-specific segregation of afferents in the tecta of three-eyed frogs. This finding suggests that the NMDA receptor, a subclass of glutamate receptors, may play a role in establishing retinal-tectal topography in normal animals.

We are investigating the frog retinal-tectal system pharmacologically in order to determine where glutamate is used as a neurotransmitter. To do this we have developed a cannulated tadpole preparation in which the circuitry between the tecta and other areas of the brain remains intact and the animal can be recorded from without the use of neuromuscular blockers which are known to affect tectal activity. Furthermore, anoxia, to which the preparation is acutely sensitive, is prevented by supplying oxygenated Ringers through two cannulae, one glued into the heart and the other positioned caudal to the tecta themselves. The optic nerve is stimulated using a bipolar electrode and the evoked field potential has a stable shape that can be recorded in the tectum for at least 8 hours. This potential consists of a large positive component followed by a large negative one. Both components are potentiated by repetitive stimulation delivered at a frequency as low as 0.5/s. The inclusion of cobalt chloride in the Ringers pumped through the cannulae reversibly blocks all of the negative component and most of the positive one, indicating that they reflect activity largely post-synaptic to the afferents. The general glutamate receptor antagonist kynurenic acid, in concentrations ranging from 0.1 mM to 1.0 mM , reversibly blocks a large proportion of both of these components in a dose-dependent manner. This result suggests that most, if not all, of the retinal ganglion cells may use glutamate as a neurotransmitter. We are currently examining the effect of APV on this preparation.

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468.9 ANTIBODY MARKERS FOR AXONS AND OLIGODENDROCYTES IN THE DEVELOPING FROG BRAIN. P. Steen, L. Kalghatgi and M. Constantine-Paton. Dept. of Biology, Yale Univ., New Haven Ct. 06511.

We have been examining the binding of two monoclonal antibodies (mabs) raised against *Rana pipiens* brain in an ongoing effort to study developing retinal processes and tectal cells. One of these mabs (LINC) recognizes most of the axons of the frog brain from early tadpole stages to the adult. On immunoblots of SDS-PAGE gels of detergent extracted frog brain LINC recognizes 2 bands in the 60-63kd range. In the tadpole tectum LINC stains some somata in layer 6, processes in the differentiated neuropil and processes within the mantle layer of caudal tectal regions prior to their invasion by retinal axons.

The second mab (OLIG) stains central and peripheral myelin and the somata and processes of the cells giving rise to these sheaths in the CNS. However it appears to bind only those oligodendrocytes that are myelinating surrounding axons. Thus in the tadpole optic tract OLIG staining is never observed among the youngest retinal ganglion cell axons adjacent to the glia limitans although soma, process and myelin staining is pronounced among the deeper, more mature retinal axons. In the tadpole tectum OLIG staining is present in both retinorecipient layer 9 and in tectal efferent layer 7 of the most rostral, mature, tectal regions. This reactivity stops abruptly in a distinct midtectal zone lying rostral to the most caudal limits of tectal differentiation and retinal axon invasion. OLIG staining has never been observed on dissociated tectal cells in culture.

LINC and OLIG binding has been examined in the contralateral visual pathway of tadpoles following unilateral optic nerve crush. LINC binding remains apparent in the pathway for at least two weeks following optic nerve crush though the appearance of bright puncta may signal degeneration. By contrast OLIG staining reveals blebbed and disrupted myelin within 6 days post-optic nerve crush. OLIG reactivity is absent in tectal layer 9 by 11 days post-crush although OLIG binding to somata, processes and myelin in the efferent layer 7 is unperturbed.

Thus LINC binding is suggestive of a cytoskeletal antigen that is relatively slow to degenerate following separation from neuronal somata while OLIG reactivity seems dependent on the availability of sufficiently mature metabolically intact axons. Supported by NIMH training grant MH18397 to PS and NIH grant EY06039 to MCP.

468.10 THE INFLUENCE OF THYROXINE ON TECTAL CELL PROLIFERATION. M. Constantine-Paton & H.T. Cline. (SPON: J. Paton) Biology Dept. Yale University. New Haven, CT. 06511.

The hormone thyroxine is responsible for metamorphosis in the frog R. pipiens. To examine the influence of thyroxine on the development of the retinotectal system, we performed a study using thymidine autoradiography to reveal patterns of cell proliferation in the tecta of tadpoles exposed to thyroxine. In one series of experiments, tadpoles (T&K st. V/VI) were raised in thyroxine (1.25 nM) for up to 2 months. Animals were injected with thymidine, sacrificed within 48 h, and their brains were processed for autoradiography. Short term thyroxine treatment (1-3 weeks) results in the superproliferation of cells in the crescent shaped proliferative zone at the caudal end of the tectum. The caudal proliferative zone includes more thymidine-labeled cells than seen in untreated animals, and the density of silver grains per cell is greater than in controls, suggesting that thyroxine may decrease the cell cycle time of proliferating cells. Longer exposure to thyroxine (~8 weeks) followed by thymidine labeling results in the incorporation of thymidine into two additional populations of cells in the rostral, relatively mature portion of the tectum: one population of cells in rostral layer 6, and a second found in the ventricular layer in sections slightly caudal to the layer 6 proliferative zone. Brains from animals with short (1/2 h) post-thymidine survival times indicate that the layer 6 cells were generated in layer 6, as opposed to being generated in the ventricular layer and migrating to layer 6. Re-examination of brains from untreated animals reveals a few thymidine-incorporating cells in layer 6 and the ventricular layer. It is commonly thought that cells generated late in development, such as those in layer 6, are exclusively glia. We are using the neuron- or glia-specific monoclonal antibodies, LINC and OLIG, described in the previous abstract (Steen et al) and commercially available antisera, in combination with autoradiography to determine whether the late-dividing layer 6 cells are neurons and/or glia. This work is supported by NIH grants EY05818 & EY06039.

468.11 SPARING OF THE VISUAL FIELD IS ASSOCIATED WITH LESS METABOLIC DEPRESSION IN THE SUPERIOR COLLICULUS OF NEONATAL VS ADULT HEMISPHERECTOMIZED CATS. D.A. Hovda, J.R. Villablanca and B.L. Shook. Ment. Ret. Res. Ctr., Depts. Anat. and Psychiat., Sch. Med., Univ. California, Los Angeles, CA 90024.

Large unilateral posterior cortical ablations in adult cats produce a complete contralateral visual field deficit as measured by visual orientation tests and this deficit can be alleviated with subsequent ablations of the contralateral superior colliculus (SC) (Sprague, J., Science, 153:1544, 1966). Here we report that removal of one cerebral hemisphere produces a similar contralateral visual field deficit in adult cats. However, the effect is attenuated if the same lesion is inflicted neonatally. Four neonatal (7-14 days of age, X = 10.6) and 4 adult cats received a left cerebral hemispherectomy. Two intact adults served as controls. After a minimum of 6 months postoperative recovery, all cats were tested via a lick suppression paradigm. Animals were food deprived and trained to drink liquid food through a licking tube while standing on a copper grid. At the licking tube animals viewed a series of 3 mm diameter light emitting diodes (LEDs) at a distance of 8 cm positioned to each side at 0^{o}, 15^{o}, 30^{o}, 45^{o}, 60^{o}, 75^{o}, and 90^{o} from the midline. A trial consisted of randomly illuminating one of the LEDs (illum. = .8 mcd, dur. = 90 nsec, peak wave length = 583 nm) followed in 1 or 3 sec by a mild foot shock which the cat could avoid by stepping off the grid onto the safe part of the chamber and thereby suppressing his licking. Using a viewing tube we verified that the cats' head and eyes were oriented to 0^{o} before each trial. All animals were tested for 1 hr/day, 5 days/wk to a minimum of 390 trials. The performance of all the cats was much better with the 1 sec delay than with the 3 sec delay with normal cats suppressing their licking to all the LEDs at all angles. Only rarely did the adult-lesioned animals suppress their licking when the stimulus was presented to the right of the midline. In contrast, the neonatal-lesioned animals consistently responded out to right 30^{o} indicating some sparing of their right visual field. Cytochrome oxidase histochemistry in 2 adult-lesioned and 2 neonatal-lesioned cats indicated that the ipsilateral SC was differentially depressed in oxidative metabolism depending on the age-at-lesion. The adult-lesioned cats showed a 16.24% depression (superficial and intermediate layers) compared to the contralateral SC. In contrast, the neonatal-lesioned animals showed only a 1.25% depression of the ipsilateral SC. Since in the present model there are no other ipsilateral neocortical areas which could account for the early lesion recovery, the differential depression of oxidative metabolism in the SC may be related to the degree of sparing of the contralateral visual field in the neonatal-lesioned cats.

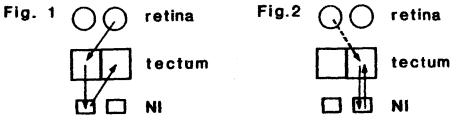
Grants USPHS HD-05958, HD 04612, and the Giannini Foundation to D.A. Hovda.

468.12 IPSILATERAL VISUOTECTAL PLASTICITY PERSISTS AFTER LESIONS OF ONE NUCLEUS ISTHMI IN XENOPUS. S.B. Udin. Dept. of Physiology, SUNY, Buffalo, NY 14214.

Visual input during development has a profound effect on binocular maps in the tectum of the frog, Xenopus. Input from the ipsilateral eye is relayed to the tectum via the opposite nucleus isthmi (NI); this ipsilateral map normally is in register with the retinotectal map from the contralateral eye. Abnormal visual input, resulting from rotation of one eye, induces the crossed isthmotectal axons to change trajectories and establish a new map in register with the retinotectal map. The major cue which aligns the maps is the correlation of visually-evoked activity from the two eyes, but we do not yet know which specific axons and dendrites are involved in these processes.

The goal of this experiment was to determine whether the activity of the retinotectal axons is sufficient to organize the crossed isthmotectal axons or whether the uncrossed isthmotectal projection also is necessary. This question was raised by the observation that contralateral input elicited by a visual stimulus reaches the tectum much earlier (about 30 msec) than ipsilateral input which arrives via a polysynaptic relay (Fig. 1). However, the input from the contralateral eye is later "echoed" by the reciprocal topographic relay shown in Fig. 2. Could the crossed axons receive their cues from this delayed activity? In order to answer this question, I examined the ipsilateral maps in frogs with unilateral lesions of the NI.

One eye was rotated by 45^{o}-150^{o} in midlarval tadpoles (stages 55-57). At stages 60-61, electrolytic lesions were made in the right NI. At 23-35 weeks after metamorphosis, the ipsilateral and contralateral projections to the right tectum were mapped. The sizes of the lesions were measured in AChE-stained sections; 44-80% of the right NI was ablated in 5 frogs; the NI was essentially intact in 3 others. In 7 of these 8 frogs, most or all of the ipsilateral map was aligned with the contralateral map. The sole exception was the map from the frog with the largest eye rotation (150^{o}). Thus, the crossed isthmotectal fibers which relay the ipsilateral visuotectal projection do not require the uncrossed isthmotectal axons in order to align with the contralateral retinotectal projection.



468.13 FUNCTIONAL SWITCHING OF VISUAL CIRCADIAN PACEMAKERS IN SPLITBRAIN CRAYFISH. B. Barrera-Mera, M. Nájar-Joa* and P. Vergara-Aragón*. Lab. of Biological Rhythms. Dept. of Physiology. Faculty of Medicine, U.N.A.M. Apdo. Postal 70250. 04510 México, D.F.

Splitbrain preparations or the simple experimental manipulation of bilaterally paired structures in nervous system have revealed some strategic operative abilities of encephalic structures with double and similar redundant function in vertebrates and invertebrates. In crayfish, the strong reciprocal interrelations among simmetrically positioned pacemaker structures, drive the complex locomotor behavior and the excitability of the nervous system. As in the cases of visual attention and many other higher functions of primates (Sperry, Science 133, 1749, 1961), the problem to separate the hemispheres of a splitbrain animal, to avoid conflict with each other, remains uncertain (Wright or Craggs, Nature 261, 580, 1976). As with the hemispheral control of visual attention in the splitbrain primates, the bilateral cyclical regulation of the circadian changes of the retinal sensitivity in crayfish (Barrera-Mera, Comp. Biochem. Physiol. 61A 427, 1978), and the activity of cat spinal respiratory motoneurons (Gromysz & Karczewski Resp. Physiol. 57, 225, 1984), results clearly incompatible. One cerebral hemiganglia dominates the behavior for a variable period of time, then the other assumes the control. Given the plastic behavior of reciprocally separated protocerebral circadian pacemakers (Barrera-Mera, Brain Res. Bull. 15, 203, 1985, we intended to explore the putative consequence of central processing of such redundant visual information, traveling to their corresponding neural networks in crayfish.

In the present communication, the behavior of circadian pacemakers was studied, while driving the crafish's retinal sensitivity. During the establishment of damping of electroretinographic oscillation in splitbrain crayfishes, we found that a bilateral synchronization of such damped oscillations, simultaneously occurred. The non compatible functional coexistence of both left and right protocerebral circadian pacemakers driving visual sensitivity in our preparations was simulated on a micro-computer. It was based on a cosin function to reproduce the cyclical events and the assumption of exponential changes in the retinal sensitivity and the phase changes that ensues after the surgical bisection of supraesophageal ganglion.

As in the visual attention process in splitbrain primates, the visual circadian phenomena here analyzed, also involves a "switching" mechanism which seems to be located in the thoraco-abdominal ganglia of these crustaceans.

468.14 DEVELOPMENT OF THE NUCLEUS OLFACTORETINALIS IN THE RAINBOW CICHLID. Sharon J. Hapner and Anne C. Rusoff. Department of Biology, Montana State University, Bozeman, MT 59717.

In many teleosts most of the cells in the nucleus olfactoretinalis project to the retina, terminating in the inner plexiform layer. Many of these cells contain an antigen specifically recognized by antibodies to FMRF-amide, a molluscan cardioexcitatory peptide (Stell, et.al. (1984) PNAS 81: 940.). We have used an immunochemical approach to trace the development of these cells in the rainbow cichlid.

In these fish development occurs very rapidly. The eggs hatch within 48 hours after being laid (when maintained at 82^{o}F.). At this time the nervous system is still very immature--retinal layers will not be visible for approximately 12 hours. Three to four days later the fry begin to swim, and they feed within 12 hours of first swimming.

Fish of different ages (before hatching to adulthood) were fixed in paraformaldehyde, frozen and sectioned at 10um. The sections were incubated with an antibody to FMRF-amide (Immunonuclear) and then reacted using the PAP procedure to demonstrate immunoreactive sites.

In the adult fish, as reported by Stell et. al., cells in the nucleus olfactoretinalis are very immunoreactive for FMRF-amide. The immunoreaction is so strong that individual cells can not be separated except at the margins of the nucleus. Axons of these cells are also strongly immunoreactive, forming a dense cluster which passes caudally toward the optic tract. Immunoreactive axons can be seen near the margin of the optic tract and optic nerve. Their terminals can be seen in the retina near the amacrine cell layer in the inner nuclear layer.

The pattern of immunoreactivity is essentially the same in young fish. We have observed intense immunoreactivity in the region of the nucleus olfactoretinalis as early as 24 hours after hatching. At this stage in development immunoreactive fibers can be traced from the nucleus olfactoretinalis into the optic stalk, through the optic fissure and into the retina. They course across the retina in the ganglion cell fiber layer adjacent to the cell bodies of the ganglion cell until they turn and cross the ganglion cell layer to enter the inner plexiform layer. Immunoreactive fibers are present in even younger fish; however, we have not yet determined the location of their cell bodies.

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469.1 TRANSMEMBRANE POTENTIAL OF FUNCTIONALLY INDENTIFIED GROUP I AND II FIBERS PRODUCED BY SEGMENTAL AND DESCENDING INPUTS IN THE CAT SPINAL CORD.
I. Jiménez*, M. Solodkin* & P. Rudomín. (SPON: Machado-Salas, J.P.) CINVESTAV, México 07000.

In spontaneously breathing cats anesthetized with nembutal we have recorded the transmembrane potential changes produced by segmental and rubrospinal inputs on 83 medial gastrocnemius (MG) afferent fibers left in continuity with the muscle. 29 fibers responded with a burst of discharges during the muscle twitch and were classified as tendon organs. 89% of these fibers had conduction velocities above 75 m/s and may be considered as Ib. in five fibers sural (SU) stimulation with pulses 2.5-20xT produced primary afferent depolarization (PAD) and either facilited, interacted linearly or occluded with the PAD produced by group I volleys to the posterior biceps and semitendinosus (PBSt) nerve. In 14 Ib fibers SU stimulation produced no PAD, but was able to facilitate (n=2), or inhibit (n=12) the PAD produced by PBSt stimulation. Only in one tendon organ fiber SU stimulation produced dorsal root reflex like activity (DRRx's). The effects of repetitive stimulation of the red nucleus (RN) were analyzed on 8 Ib fibers. RN stimulation produced PAD in 3 fibers, no transmembrane potential changes in other 3 fibers, and probably PAH in two Ib fibers. 54 fibers showed a silent period during the muscle twitch and were clasified as from muscle spindles. SU nerve stimulation produced PAD in one spindle afferent, DRRx's in 15 spindle afferents and no transmembrane potential changes in 38 spindle afferents. However, SU stimulation inhibited the PAD produced by PBSt group I volleys in 26 fibers and facilited the PAD in 4 fibers. 86% of the fibers had conduction velocities above 75 m/s and were considered as group Ia. 9 fibers had conduction velocities below 70 m/s and could well be from group II. In 4 of them SU stimulation produced DRRx's and in 2 SU stimulation produced no PAD but inhibited the PBSt induced PAD. In the other fibers SU stimulation had no effect. Stimulation of the RN produced no PAD in six Ia fibers. In three of them RN stimulation had no effect on the PAD induced by PBSt stimulation, and in one fiber the PAD was facilited. These results are in agreement with previous findings showing that SU stimulation may either produce PAD or inhibit the PAD of group Ib fibers (J. Neurophysiol.56:987,1986). They suggest also that group II fibers have transmembrane potential responses resembling those seen in Ia rather than Ib fibers, in contrast with what has been reported by other investigators (J. Neurophysiol. 26:1,1963) Partly supported by grants NIH NS09196 and CONACyT PCCBBNA 021782.

469.2 A comparison of homonymous and heteronymous connectivity between Ia and spindle group II fibers and motoneurons in the spinal cord of the cat. H.-R. Lüscher and U.Vardar* (SPON. M. Wiesendanger). Department of Physiology, University of Berne, CH-3012 Berne, Switzerland.

Since the motoneurons (MNs) supplying different muscles (e.g. medial and lateral gastrocnemius) are intermixed to a great extent in the motor column of the spinal cord, the spindle afferent fibers from the muscles must be guided very precisely to establish the connections that mediate their localized stretch reflex. Cell surface molecules have been proposed as a chemical marker for recognition between the afferent fibers and the MNs. In this study we could demonstrate that the sizes of the MNs and muscle afferent fibers and their topographic relations to each other play decisive roles in determining connectivity.

Multi-unit spike triggered averaging was used to determine connectivity between Ia and spindle group II fibers from the medial gastrocnemius muscle and the MNs innervating the medial and the lateral gastrocnemius-soleus muscle. As many as 288 possible connections between 24 MNs and 12 afferent fibers were studied in single, acute experiments. The influence of morphological and topographical factors as well as of MN spezies on functional connectivity were analysed. The probability that a MN would receive functional connections from a given population of afferent fibers was related to its size and its proximity to the spinal entry level of the afferent fibers. The faster the axonal conduction velocity of the MN and the closer its location to the entry zone of the afferent fibers the greater was its receptivity. This relationship was qualitatively similar for homonymous and heteronymous MNs. 58 % (233/399) of the Ia and group II afferents (combined) had functional connections with homonymous MNs, 32 % (75/234) with heteronymous MNs. However, homonymous and heteronymous MNs of similar sizes were equally likely to receive functional connections when located at the same craniocaudal level. Differences in the locations and sizes of homonymous and heteronymous MNs evidently account largely for the observed overall differences in homonymous and heteronymous connectivity. Species recognition apparently play only a very minor role in the formation of connections, at least for the close synergists studied.

The observed pattern of connectivity suggests that connections are formed without further constraints than those given by the architecture and topographical relations of the neural elements involved. (Supported by the Swiss National Science Foundation No. 3.265-0.85)

469.3 SLOWLY ADAPTING PRESSURE RECEPTOR INPUT TO GAMMA MOTONEURONES REVEALED BY CROSS CORRELATION OF UNIT ACTIVITY IN THE CAT.
P. H. Ellaway* and N. J. Davey* (SPON: M.C. Wetzel). Dept. of Physiology, University College London, London WC1E 6BT, England.

The background discharges of gamma motoneurones to the gastrocnemius muscle in the cat exhibit a pronounced degree of short term synchrony following complete spinal section (T9). The degree of synchrony is enhanced during excitation of gamma motoneurones by non-noxious, mechanical stimulation of the skin of the ipsilateral heel (Ellaway, P. H. and Murthy, K. S. K., Quart. J. Exp. Physiol., 70: 233, 1985). This suggests that the activity of certain mechanoreceptors exerts a strong influence on gamma motoneurones through shared synaptic inputs.

To investigate this coupling, and to identify the afferents responsible, we have recorded the discharges of single afferent neurones and cross correlated their activity with that of single gamma motoneurones in the decerebrate spinal cat. Single afferents from the sural nerve field of the heel were recorded in the dorsal root ganglion (DRG). Continuity with the spinal cord was confirmed by averaging a recording from the dorsal root triggered by the DRG spikes. Discharges of gamma motoneurones were recorded from filaments of the gastrocnemius muscle nerve.

Cross correlations between the discharges of gamma motoneurones and rapidly adapting (RA) afferents innervating hairs never showed any coupling (n=15). In contrast, cross correlograms of the discharges of slowly adapting (SA) mechanoreceptors and gamma motoneurones showed a peak of increased probability of motoneurone discharge in 12 out of 27 instances. The peaks in the correlograms were delayed with respect to the afferent discharge and had widths at half maximum in the range 2 to 7 ms (mean 3.7 ms). The afferents responsible had conduction velocities in the range 53 to 75 m/s (plus one at 29 m/s). The gamma efferent axon conduction velocities ranged from 19 to 31 m/s. Central delays of the gamma motoneurone response to arrival of an afferent impulse had a mean value of 4.1 ms (range 3 to 6.5 ms).

Typically, the afferent response to maintained pressure was a sustained, irregular discharge with a prominent dynamic component: there was no background firing. This pattern is typical of slowly adapting type 1 receptors innervating Merkel cells.

We conclude that type 1 SA afferents from the sural field have a tight coupling with gamma motoneurones of the closely related gastrocnemius muscle. Although the long central delay implies an oligosynaptic pathway, the correlation between impulses in individual primary afferent and efferent neurones suggests, rather, that a single interneurone may be involved.

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469.4 THREE-DIMENSIONAL DIRECTIONAL SENSITIVITY OF NECK MUSCLE SPINDLE RESPONSES TO HEAD ROTATION. V.J. Wilson, J. Kasper*, B.J. Yates and R.H. Schor. The Rockefeller University, New York, NY 10021.

We have recently studied the response of muscle spindle afferents in the C2 dorsal root ganglion (DRG) to neck rotation in roll-pitch planes (Chan et al., J. Neurophysiol. 57, 1987). We have now expanded the study by adding neck rotation in the yaw plane, making possible determination of the most effective direction of sinusoidal stretch (response vector) for each afferent.

Using floating electrodes, we recorded from the left C2 DRG of decerebrate, paralyzed cats during head rotations. Input to the ganglion was surgically restricted: afferents were therefore in ventral and ventrolateral perivertebral muscles, as well as in sternomastoid and, to a lesser extent, clavotrapezius. Spindle afferents were identified by their vigorous excitation by intravenous succinylcholine. Three-dimensional (3-D) response vectors were either calculated from the gains in roll, pitch and yaw, or approximated from the 2-D vectors in the roll-pitch and pitch-yaw planes determined by "wobble" stimuli (Schor et al., J. Neurophysiol. 51, 1984). Each afferent's linearity was then studied with 2-D planar stimuli oriented as close as possible to its vector, using sinusoids of 0.5-5 deg at 0.2 Hz. Dynamic index was measured from response to similarly oriented trapezoidal stretch.

3-D response vectors were determined for 44 spindle endings. Yaw to the right, roll right ear down, or nose-up pitch were effective stimulating directions for most afferents. Few were dominated by one direction of stretch; instead at least two directions (i.e. pitch and yaw) evoked substantial responses. As in the earlier study we observed afferents in three response categories: 21 type A (non-linear, high gain and dynamic index), 12 type B (fairly linear, low gain and dynamic index), 11 ambiguous (intermediate properties). The coefficient of variation of spontaneous firing rate of type B spindles was always low (<0.1), while cv values of type A and ambiguous receptors covered a wide range. Five endings had shown type B behavior in response to roll-pitch stimulation, but their response became more dynamic during pitch-yaw stimulation. It seems likely that the remaining 12 type B receptors include secondary muscle spindle endings; type A receptors have primary-like properties. (Supported by NIH (NS02619), (NS17808), NASA (NSG2380) DFG fellowship KA694/1-1 (J.K.), and NIH fellowship NS08050 (B.J.Y.).

469.5 DESCENDING PROJECTIONS OF NECK MUSCLE SPINDLE AFFERENTS IN THE DORSAL COLUMNS. B.J. Yates, J. Kasper*, and V.J. Wilson. The Rockefeller Univ., New York, N.Y. 10021.

The deep muscles surrounding the vertebrae have a rich content of muscle spindles (Bakker and Richmond, J. Neurophysiol. 48, 1982). Recent studies have suggested that these perivertebral spindles provide part of the afferent input to the tonic neck reflex (Chan et al., J. Neurophysiol. 57, 1987). It has also been proposed that medially located propriospinal neurons in C4 and C5 form part of the circuitry of this reflex. Propriospinal neurons located in this region, many of which have crossed projections to segments containing forelimb motoneurons, are modulated by neck rotation (Brink et al., J. Neurophysiol. 54, 1985). It is not known how inputs from neck muscle afferents, including spindles, which enter the spinal cord through the upper cervical dorsal roots, are relayed to neurons in C4 and C5. We have recently attempted to test the hypothesis that primary neck muscle spindle afferents themselves may carry some of the inputs to the propriospinal neurons.

We recorded extracellularly from spontaneously active neurons in the C2 dorsal root ganglion in decerebrate, paralyzed cats using floating electrodes. Afferent input to the ganglion was reduced by denervation of the neck skin and most of the large dorsal neck muscles. Most of the spontaneously active afferents were classified as spindle afferents, and the properties of the cells were studied using previously described procedures (Chan et al., J. Neurophysiol. 57, 1987; preceding abstract). An attempt was made to antidromically drive the afferents using a movable ball electrode placed on the dorsal columns. If antidromic stimulation at mid C3 was successful, the electrode was moved caudally stepwise. This continued until the afferent could no longer be driven. 23/50 of the tested cells could be driven antidromically from C3 or more caudal levels; 7 could only be driven as far caudally as C3, 9 could be driven from C4 but no further caudally, and 7 could be driven from C5. The conduction velocities along these descending projections were mainly between 20 and 30 m/sec; the mean +/- SEM was 26.7 +/- 2.7 m/sec. Latencies to C4 ranged from 0.7 to 1.4 msec. Both afferents with primary-like and secondary-like characteristics had long descending projections in the dorsal columns. In addition, the afferents with long descending branches could be best modulated by a wide range of directions of neck rotation.

These results show that C2 neck muscle spindle afferents project to more caudal segments containing propriospinal neurons believed to relay neck inputs to forelimb motoneurons. Supported by grants from NIH (NS02619) and NASA (NSG2380). B.J.Y. was supported by NIH fellowship NS08050 and J.K. was supported by DFG fellowship KA694/1-1.

469.6 COLLATERALIZATION OF VESTIBULOSPINAL AXONS FROM DEITERS' NUCLEUS TO LUMBAR CORD AND LOCUS COERULEUS IN RATS AS DEMONSTRATED BY RETROGRADE TRANSPORT OF HRP AND FLUORO-GOLD. S.J. Fung, V.K. Reddy*, H. Zhuo* and C.D. Barnes, Dept. of VCAPP, Washington State University, Pullman, WA 99164.

Our recent anatomical findings have determined a significant input to the cat and rat locus coeruleus (LC) arising mostly from the ipsilateral Deiters' neurons. As the latter cells also contribute inputs to the descending lateral vestibulospinal tract, it is expected that some of the Deiters' neurons must innervate the spinal cord and the LC through axon collateral branches. The present study was undertaken to demonstrate these connections, viz. lateral vestibulospinal and Deiters'-LC projections, by applying two separate retrograde tracers to the above-mentioned axonal terminal fields of the Deiters' nucleus.

Adult Sprague-Dawley rats were used in this study. Under Nembutal anesthesia (60 mg/Kg, i.p.), horseradish peroxidase (HRP) and Fluoro-Gold were pressure-ejected via glass microelectrodes into the lumbar cord and the LC, respectively, over two surgical sessions. Six microelectrode penetrations were made bilaterally into the fifth or sixth lumbar segment while at least 1 µl of 30% HRP (type VI, Sigma) was delivered to each animal. After 24 hr. the animal was reanesthetized and a single ejection of 4% Fluoro-Gold (Fluorochrome, CO) was achieved via a microelectrode aimed stereotaxically at the left LC (1.1 mm lateral to the sagittal suture, 1.2 mm caudal to the lambda suture, and 5.5 mm below the brain surface). Each animal was then perfused after 24 hr. with warm saline, cold 3.8% paraformaldehyde in 0.1M phosphate buffer, and 30% sucrose solution. Coronal sections of the brainstem were cut with a cryostat at a thickness of 30 µm. Tissue sections were reacted with TMB method for visualization of HRP labeling. Fluoro-Gold labeling was screened under the fluorescence microscope by using ultraviolet excitation.

The present analysis has included four animals containing substantial deposits of Fluoro-Gold at the LC area with minimal spread to the adjacent dorsolateral pontine structures. From these animals, double-labeled neurons were consistently found on both sides of the LC injection site, among the HRP-labeled Deiters' neurons. Double-labeled Deiters' neurons of various sizes were distributed randomly with respect to the single HRP-labeled lateral vestibulospinal tract cells. Preliminary counting revealed that approximately 10% of the spinally projecting Deiters' neurons also terminate at the ipsilateral LC through axon collaterals. These data support a possible role of the LC on central processing of vestibular impulses. Supported by NIH grant NS24388.

469.7 LOCATION OF AFFERENT TERMINALS IN MONKEY SPINAL CORD AND BRAIN STEM AS REVEALED WITH CHOLERA TOXIN - HRP INJECTED INTO A FOREARM MUSCLE NERVE. J.S. McIntosh, E.M. Schmidt and M. Kalia. Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20892 (JSM and EMS), and Department of Pharmacology, Thomas Jefferson University, Philadelpia, PA 19107 (MK).

Understanding the organization of the motor system requires knowledge of the anatomical location of the motoneuron pool supplying a single muscle, and the spinal cord and brain stem locations where terminations of sensory fibers from the same muscle occur. HRP conjugated with cholera toxin (CTHRP) provides a sensitive marker for anterograde tracing of pathways and their terminations along with retrograde transport for labeling motoneurons. Levine et al. ('86) reported that CTHRP was not transported transynaptically, thus only the first order efferent and afferent pathway will be labeled.

Under sodium pentobarbital anesthesia and sterile operating conditions, two Macaca cynomologus monkeys were prepared for pressure injections of CTHRP. Two ul of CTHRP were injected through a glass micropipette into one of the nerve branches innervating flexor carpi ulnaris. After recovery from surgery, the animals were allowed to survive for 5 days. The animals were deeply reanesthetized with sodium pentobarbital and perfused transcardially with saline followed by para-gluteraldehyde fixative. Frozen serial sections of the spinal cord, dorsal root ganglia, and brain stem were cut at 75 um and processed with TMB. Serial reconstructions were carried out on a computer coupled microscope system.

The motoneuron pool for FCU, labeled with CTHRP, was located in the C8 - T1 segments of the spinal cord. The location was the same as previously reported with horseradish peroxidase conjugated with wheat germ agglutin (WGAHRP) nerve injections (Schmidt and McIntosh '85). However, CTHRP fills the entire dendritic field of the motoneurons and provides a more complete view of the extent of the motoneuron pool than was seen with WGAHRP. The labeled afferents entered the spinal cord through the C8 and T1 dorsal root ganglia (DRG). The labeled cell bodies in the DRG were distributed throughout the ganglia suggesting a lack of somatotopy for an individual muscle nerve. Afferent fibers could be traced through the dorsal column and dorsal horn to the brain stem. Swellings occurred at many points along the fibers in the dorsal horn suggesting synaptic terminals. Terminal fields were located in the dorsal horn throughout the entire length of examined spinal cord (C2-T1). The terminal fields in the brain stem formed a mosaic rather than a continuous distribution throughout the dorsal column nuclei.

CTHRP is a useful tool in tracing muscle afferent terminal fields and is appropriate for use in the monkey.

469.8 **TRANSNEURONAL TRACERS REVEAL CONNECTIVITY OF RAT PENILE MOTONEURONS.** R.D. Rose, J.T. Erichsen and W.F. Collins III. Dept. Neurobiology & Behavior, SUNY, Stony Brook, NY 11794

Motoneurons (MNs) which innervate striated muscles involved in penile reflexes in the adult male rat are located in the dorsomedial (DM or SNB) and dorsolateral (DL) nuclei, two motor nuclei in the lumbosacral spinal cord. Several retrograde labeling studies, using horseradish peroxidase (HRP), have elucidated the organization of DM and DL as well as the number and size distribution of the MNs within them (Breedlove & Arnold, 1980; Schroder, 1980; McKenna and Nadelhaft, 1986).

In the present studies, we have used two putative transneuronal tracers (wheat germ agglutinin [WGA] and tetanus toxin c-fragment [TTC]) to investigate connectivity among MNs involved in penile reflexes. MNs were retrogradely labeled following intramuscular injection of tracer (10ul of 0.1% WGA or 10 ul of 15% TTC). Procedures for the immunohistochemical localization of the tracer have been described (WGA: Rose and Collins, 1985; TTC: Evinger and Erichsen, 1986). Studies utilizing HRP or WGA have yielded similar results in hindlimb motoneuron systems. In contrast, striking qualitative differences were observed in the distribution of WGA and TTC labeling of penile motoneurons compared with previously reported HRP labeling. For example, HRP labeling of bulbospongiosus MNs (Schroder, 1980; McKenna and Nadelhaft, 1986) resulted in labeled MNs exclusively in the ipsilateral DM (=SNB). Unilateral WGA injections into dorsal (LA) or medial/lateral (BC) bulbospongiosus muscles, however, resulted in up to 50% more labeled MNs in the ipsilateral DM as well as labeled MNs in the ipsilateral DL and the contralateral DM. Similarly, unilateral TTC injections of LA resulted in densely labeled MNs in ipsilateral DM and DL and contralateral DM, but TTC was also found in the contralateral DL. Both WGA and TTC labeled inter- and intra-nuclear thickets of dendritic processes that traverse between labeled MNs.

The patterns of WGA and TTC labeling observed in the present studies may be the result of transneuronal labeling and suggest a marked connectivity among ipsilateral as well as contralateral penile MNs. Such connectivity may serve to help synchronize the activity of these MNs during copulation.

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469.9 DIFFERENCES IN THE MORPHOLOGY AND FREQUENCY OF AXON TERMINALS ON PROXIMAL AND DISTAL DENDRITES OF NECK MOTONEURONS IN THE CAT. P.K. Rose and M. Neuber*, Department of Physiology, Queen's University, Kingston, Ontario, K7L 3N6.

It is now well established that most of the surface area of spinal motoneurons which is available for synaptic contact lies on the dendritic tree. This large area is arranged in an organized fashion which may provide a substrate for segregation of inputs from different sources. In the present experiments the morphology and frequency of axon terminals contacting different regions of neck motoneurons were compared. Motoneurons were identified electrophysiologically and stained intracellularly with HRP. The position of each axon terminal was determined by matching the relative position of dendrites seen at the electron microscopic level with drawings of the dendritic tree constructed at the light microscopic level.

The axon terminals on two motoneurons innervating biventer cervicis have been examined in detail. All axon terminals of one of these motoneurons were located on dendrites caudal to the soma at least 540 um from the soma. Axon terminals on the soma and proximal dendrites (up to 240 um from the cell body) were studied in the other cell. The percentage of postsynaptic membrane in contact with axon terminals and estimates of synaptic density for the somatic, proximal dendritic, and distal dendritic regions are summarized below.

	Soma	Proximal Dendrites	Distal Dendrites
Perimeter in contact with axon terminals	71%	67%	22%
Axon terminal density (number/100 um^2)	11.9	10.7	6.7

These differences were associated with differences in the morphology of axon terminals terminating on different regions. 72% of axon terminals on the soma and 57% of axon terminals on proximal dendrites contained either spherical or pleomorphic vesicles which were tightly packed in clumps. In contrast, these boutons were rare (8%) on distal dendrites. The most common (42%) type of axon terminal on distal dendrites contained a dense collection of evenly distributed spherical vesicles. This type of axon terminal was rarely observed on proximal dendrites (6%) and the soma (1%).

The differences in axon terminal density and morphology may be attributed to intrinsic differences between the two cells or they may be related to the zone of the cell on which the axon terminals were found (Supported by MRC).

469.10 POST-TETANIC POTENTIATION OF RECURRENT IPSPs IN CAT MOTONEURONS. M. D. Binder and A. D. Lindsay*. Dept. of Physiology & Biophysics, Univ. of Washington, Seattle WA 98195.

During the course of our analysis of the distribution of steady-state, recurrent IPSPs within the cat triceps surae motoneuron pools, we noticed a marked potentiation of the amplitudes of the maximal recurrent IPSPs produced by single shocks delivered to synergist muscle nerves. In light of the recent, extensive analyses of post-tetanic potentiation (PTP) in the Ia afferent-motoneuron synapse (Luscher et al., Nature 282:859, 1979 and J. Neurophysiol. 49:269, 1983; Lev-Tov et al., J. Neurophysiol. 50:379, 1983; Collins et al., J. Neurophysiol. 52:980, 1984)), we decided to examine the PTP of recurrent IPSPs in a systematic manner. Of particular interest was the question of whether PTP in this system might be differentially distributed within a pool of motoneurons as is the case for PTP in the Ia afferent-motoneuron synapse.

Intracelluar recordings were made from triceps surae motoneurons of barbiturate-anesthetized cats in which the ipsilateral dorsal roots had been sectioned. The motoneurons were identified by antidromic invasion and measurements were made of their input resistances and rheobases. Recurrent IPSPs were evoked by single, supra-maximal electrical shocks delivered to the synergist muscle nerves. Control IPSPs were recorded at a stimulation frequency of 1 Hz. Subsequently, the synergist muscle nerves were stimulated at 500 Hz for ten seconds and the potentiated response was measured 15 sec. after the end of the tetanization. Thus far, clear evidence of potentiation has been observed in each motoneuron studied. When expressed with respect to the control IPSPs, the extent of potentiation ranged from 12% to nearly 100%. As expected, the amplitudes of both the control and potentiated recurrent IPSPs were related to motoneuron input resistance. Moreover, as is the case for the Ia afferent-motoneuron synapse (e.g., Luscher et al., Nature 282:859,1979), the degree of potentiation was greater in medial gastrocnemius motoneurons with low input resistances than in those with high input resistances. This differential distribution of PTP within a motoneuron pool implicates the Renshaw cell-motoneuron synapses in the potentiation since the same motor axon collateral- Renshaw cell synapses are common to each trial. Further, these results provides another example of the "shaping" of synaptic input by the characteristics of the post-synaptic cell. Supported by NIH grant NS 22417.

469.11 ANALYSIS OF STEADY-STATE RECURRENT IPSPs AND THEIR UNDERLYING EFFECTIVE SYNAPTIC CURRENTS IN CAT MOTONEURONS. A. D. Lindsay* and M. D. Binder (SPON: W.E. Crill). Dept. of Physiology & Biophysics, Univ. of Washington, Seattle, WA 98195.

Systematic variation in the amplitudes of synaptic potentials is thought to be the major mechanism underlying the orderly recruitment of motoneurons innervating skeletal muscles. However, the extent to which this variation can be attributed to differences either in the intrinsic cellular properties that determine the input resistance of the motoneurons or in the factors that determine the effective synaptic current reaching their somata remains unresolved. Measurements of motoneuron input resistance and the amplitudes of the synaptic potentials produced by several different input systems have generally revealed that these parameters covary, but inferences based on this covariance have been limited by the absence of measurements of the actual input, i.e. the effective synaptic current. We have previously described a simple current injection technique, a variant of the standard voltage clamp, that provides these missing data under steady-state conditions (Heckman and Binder. Neurosci. Abstr. 11: 402, 1985; Heckman et al., Neurosci. Abstr. 12: 247, 1986). In our most recent series of experiments, we have modified our protocol to examine the distribution of recurrent IPSPs and their underlying effective synaptic currents in the cat triceps surae motoneuron pools. Steady-state recurrent IPSPs were produced by applying supra-maximal electrical stimuli to the synergist muscle nerves at 100 Hz (ipsilateral dorsal roots cut) for 1 sec. Small amounts ($\pm$ 1 to 3 nA) of current were injected into the cell through the recording microelectrode for 500 ms before and 500 ms during the steady-state IPSP. This procedure provided measurements of both the input resistance (Rn) of the motoneurons (including any significant changes in the resting value of Rn caused by the tonic activation of synaptic conductances) and the amplitude of the steady-state recurrent IPSP. We repeated this procedure using three different levels of injected current so that we could determine the reversal potential for the recurrent IPSP in each motoneuron and thus, normalize our synaptic potential measurements to a standard resting potential. The effective synaptic current could then simply be calculated from Ohm's law. We have found that the amplitudes of the steady-state recurrent IPSPs covary with Rn. Moreover, as is the case for the Ia afferent input system, the underlying effective synaptic currents generated by Renshaw cells appear to be greater in triceps surae motoneurons with high input resistances than in those with low input resistances. Supported by NIH grant NS 22417.

469.12 PRIMED VS UNPRIMED: COMPARISON OF CAT SPINAL MOTONEURON DISCHARGE CHARACTERISTICS DURING THE INJECTION OF HAVERSINE DEPOLARIZING CURRENT WITH AND WITHOUT PINK NOISE. J.M. Spielmann* G.A. Robinson, C.S. Yuan* R.M. Reinking* and D.G. Stuart. (SPON: P.E. Pickens). Dept. of Physiology, Univ. of Arizona, Tucson, AZ 85724.

The synaptic noise recorded intracellularly in motoneurons is conditional on the type of anesthesia used during experimentation, with pentobarbital usually reducing the membrane's excitability the most. To determine the effects of membrane excitability on motoneuron discharge characteristics under pentobarbital anesthesia, we injected repetitive haversine depolarizing currents with and without the addition of pink noise. Surgical preparation consisted of a lumbar laminectomy and a complete left hip and hindlimb denervation. Our intracellular recording techniques have been described previously (Watt et al., J. Neurophysiol. 39:1375, 1976). Haversine current was injected repetitively at 3.85 Hz for at least 90 s at current levels for a single discharge (threshold(T)) and 2T. In addition, at both current levels, discharge properties were examined with and without pink noise (+/- 1-3 mV membrane shift, peak to peak). Motoneurons were characterized, regardless of species, as fast (F) or slow (S) as described previously (Zengel et al., J. Neurophysiol. 53:1323, 1985). Discharge properties were analyzed for minimum spike interval, burst duration, time to first spike, number of spikes, peak and average frequencies over 90 s of current injection. Results indicate that type S motoneurons respond to the addition of noise by increasing the number of spikes whereas type F motoneurons do not. With regard to the change in the number of spikes over time (accommodation), F motoneurons accommodated to a greater degree than type S motoneurons. Supported by USPHS grants NS 07888 and HL 07249.

469.13 COMPARISON OF CAT SPINAL MOTONEURON DISCHARGE CHARACTERISTICS DURING INJECTION OF HAVERSINE AND RECTANGULAR DEPOLARIZING CURRENTS. R.M. Reinking* G.A. Robinson, J.M. Spielmann* C.S. Yuan* and D.G. Stuart. (SPON: L.L. Rankin). Dept. of Physiology, University of Arizona, Tucson, AZ 85724.

Traditionally, current injection using a rectangular waveform has been used to study the discharge characteristics of motoneurons (e.g. Kernell, Acta. physiol. scand., 65:65, 1965). Recent evidence suggests that another waveform best resembles the net depolarizing pressure imposed on motoneurons (Hoffer et al., J. Neurophysiol., 57:530, 1987). Preliminary to a neuromuscular-fatigue study on the adaptive properties of motoneurons to repetitive current injections, we considered it necessary to compare motoneuron discharge characteristics to rectangular- and haversine-shaped currents, the latter approximating the locomotor command. Surgical preparation consisted of a lumbar laminectomy and complete left hip and hindlimb denervation, all under pentobarbital anesthesia. Our intracellular recording techniques have been described previously (Watt et al., J. Neurophysiol., 39:1375, 1976). Each waveform test was divided into two categories based on pulse duration and off time (60/200 msec and 500/225 msec respectively) representing different types of depolarizing pressure. In 3 cats, 37 lumbosacral motoneurons were characterized, regardless of species, as fast (F) or slow (S) as described previously (Zengel et al., J. Neurophysiol., 53:1323, 1985). Discharge properties were analyzed for minimum spike interval, burst duration, time to first spike, number of spikes, peak and average frequencies at current levels for a single discharge (threshold (T)), 1.5T, 2T and 3T. The analysis to date suggests that there are no differences in the measured parameters, between waveform types, for type F and S motoneurons. However, the temporal pattern of spike generation, which is important for the optimalization of muscle-force development (Zajac and Young, J. Neurophysiol., 43:1206, 1980) is still being analyzed. Supported by USPHS grants NS 07888 and HL 07249.

469.14 RETROGRADE LABELING OF MOTORNEURONS INNERVATING MUSCULATURE ASSOCIATED WITH THE FOAM GLAND IN MALE JAPANESE QUAIL. C.M. Seiwert and E. Adkins-Regan. Psychology Department, Cornell University, Ithaca, NY 14853.

The dorsal proctodeal gland (foam gland) of Japanese quail (*Coturnix c. japonica*) is a sexually dimorphic structure located in the dorsal cloaca immediately posterior to the vent and directly beneath the large circular sphincter ani muscle. The gland secretes a fluid which is whipped into a stiff foam, presumably by the undulation of the sphincter ani muscle (Klemm, R.D. et al., J. Morph., 141:171-184, 1973). Although its function is unknown, the foam is transferred to the female during copulation and is also deposited during defecation.

The purpose of this study was to locate and characterize the motoneurons responsible for the innervation of the sphincter ani muscle. Horseradish peroxidase (HRP, 8.5 mg/20 µl) was injected into the target muscle of adult male Japanese quail (n=5, average weight 162 gm). Subjects were sacrificed and perfused 48 hours later. Spinal cords and cloacas were sectioned at 40 µm. Labeled cells were visualized with a modification of the tetramethyl-benzidine technique and counterstained with neutral red.

Examination of cloacal musculature confirmed that HRP was restricted to the sphincter ani. Labeled motoneurons with multiple primary dendrites were observed in the 9th and 10th synsacral segment of the spinal cord, in the region of the rhomboid fossa. Labeled cells were confined to a nucleus located in Area IX of the lateral horn. Axons exiting through the ventral horn were visible on many of the labeled cells.

Japanese quail are unique amoung birds in their possession of an active foam gland, though smaller, less active glands are present in other species of quail (Schleidt, W.M. and Shalter, M.D., Ibis., 114:558, 1972). Although the foam gland and sphincter ani are present in both sexes, the gland and muscle are small in the female in comparison to the male, and the female produces no foam (Klemm, R.D. et al., J. Morph., 141:171-184, 1973). Foam production, gland size, and muscle size are androgen-dependent (Nagra et al., Anat. Rec., 133:415, 1959) and are positively correlated with strutting and crowing, two sexually dimorphic behaviors (Adkins, E. and Adler, N.T., J. Comp Physiol. Psych., 81:27-36, 1972). These sex differences in peripheral structure may be reflected in differences in the central nervous system (CNS), allowing an analysis of the development of such differences. Further, this system may lend itself to investigations of the development of species differences in CNS organization, thus providing insight into relationships between brain ontogeny and evolution. (Supported by NSF #BNS 8412083)

469.15 MORPHOLOGY OF CAT SACROCAUDAL MOTONEURONS. S.M. Bailey*, L.A. Ritz and G.W. Sypert. Departments of Neurosurgery and Neuroscience, University of Florida Health Center, Gainesville, Fl. 32610.

While much is known about the morphology of motoneurons innervating appendicular musculature, little is known about the dendritic organization of motoneurons projecting to axial musculature. We have described the projection patterns of cutaneous and muscle primary afferent fibers emanating from the tail. We are extending our studies of the sacrocuadal spinal cord to include motoneurons by using intracellular injection of horseradish peroxidase (HRP).

Adult cats were anesthetized with Nembutal and the sacrocaudal spinal cord exposed. S3, Cal and/or Ca2 ventral roots were placed on stimulating electrodes. Motoneurons were identified by a short, antidromic conduction latency. An intrasomatic electrode position was ascertained by a d.c. shift in membrane potential, by the size of the action potential and by the presence of the IS/SD break on the action potential. HRP was injected with positive current for a total of 60 to 120 na-min and revealed by Hanker-Yates histochemistry.

Preliminary observations, based on intrasomatic injections of 21 antidromically-identified motoneurons, suggest that: 1) the average diameter of the cell bodies ranged in size from 35 to 63 micrometers (mean of 46); 2) there were five to eight primary dendrites; 3) dendrites were observed throughout the ipsilateral ventral horn and occasionally extended into the base of the ipsilateral dorsal horn; 4) every motoneuron had dendrites reaching into the ipsilateral ventral, lateral and dorsolateral white matter; 4) dendrites crossed ventral, or occasionally dorsal, to the central canal and projected into the contralateral ventral horn, in some cases reaching the contralateral ventral and lateral white matter; 5) dendrites extended up to 2300 micrometers rostrocaudally, 1580 micrometers dorsoventrally and 1950 micrometers mediolaterally.

These observations suggest that sacrocaudal motoneurons: 1) have cell bodies which are slightly smaller than those of the lumbar cord; 2) have dendrites with considerable spread ipsilaterally, throughout the ventral gray and white matter; 3) have dendrites which usually reach into the contralateral ventral gray and white matter.

(Research supported by NS23683 and the Veterans Administration Medical Research Service.)

469.16 MICROELECTRODE STUDIES OF LARYNGEAL MOTONEURONS IN THE NUCLEUS AMBIGGUS OF THE AWAKE VOCALIZING MONKEY. David L. Zealear and Charles R. Larson. Department of Otolaryngology, Vanderbilt University Medical Center, Nashville, Tennessee 37232 and Department of Speech and Language Pathology, Northwestern University, Evanston, Illinois 60201.

The somatotopic organization and functional roles of laryngeal motoneurons in swallowing, respiration, and vocalization were studied in the awake fasicularis monkey. Animals were first trained to produce two types of calls for food rewards: a "coo" and a "bark". Following sterile implant sugery, physiological sessions were conducted on each animal. The nucleus ambiguus was targetted using stereotaxic coordinates and located with a microelectrode by evoking an antidromic field potential within the nucleus. Isolated recordings from neurons were studied with respect to their firing pattern with behavior. Laryngeal motoneurons were identified using standard criteria, including antidromic invasion, frequency following, and collision. A motoneuron was microstimulated while recording EMG responses to determine the muscle it innervated.

The characteristics of approximately 50 well isolated neurons located within the thyroarytenoid representation of the nucleus (i.e. middle third) were examined in detail. Three types of neurons were observed. The most prominent was a nonrespiratory type of motoneuron, which was recruited phasically for both swallowing and vocalization. Although rare, a second type of motoneuron was also identified. It was recruited tonically for expiration, but was inhibited during both swallowing and vocalization. The third type, found abundantly, was an inspiratory interneuron, which presumably inhibited motoneurons during inspiration. The finding of two types of motoneurons is consistent with previous observations of two types of motor units (FR,S) and muscle fibers (IIA,I) comprising the muscle. The greater abundance of phasic motoneurons correlates well with the larger proportion of FR motor units and type IIA muscle fibers within the muscle and implicates the muscle in rather ballistic laryngeal functions such as airway protection and pitch modulation.

469.17 NON-LINEAR INTERACTION BETWEEN BACKGROUND SYNAPTIC NOISE AND Ia SINGLE FIBER EPSPs EVOKED IN SPINAL MOTONEURONS. M. Solodkin*, O. Ruiz de Leon*, L. Zamora*, I. Jimenez*, W.F. Collins III, L.M. Mendell and P. Rudomin. (Spon: J. Alvarez-Leefmans). CINVESTAV, Mexico 07000 and SUNY-Stony Brook, NY 11794.

During steady stretch of the medial gastrocnemius (MG) muscle in anesthetized cats we noted that single fiber MG Ia-EPSPs, when preceded at a critical interval (2 ms) by large (500-700 uV) spontaneous EPSPs, were smaller (65 ±21%) and had a faster falling phase (decrease in half width by 42±9%) than control Ia-EPSPs, even after correcting for a shifting baseline (n=7). To understand these non-linear interactions we analyzed the fluctuations of Ia-EPSPs evoked in homonymous motoneurons when the muscle was slack or stretched (low and high background synaptic noise, respectively). At some connections we noted an increase in variance of membrane potential above baseline levels during the Ia-EPSP when the muscle was slack, but not when it was stretched. If synaptic noise interacts linearly with evoked Ia-EPSPs, theory predicts that their fluctuations are independent of the magnitude of background noise. To understand further how these non-linear interactions affect Ia-EPSP fluctuations we developed a model that allows addition of various amounts of noise to a fluctuating signal and subsequent extraction of its amplitude distribution with a deconvolution procedure. Assuming linear addition and a constant signal (i.e. amplitude distribution with 1 peak), the same signal was extracted regardless of the amount (i.e. variance) of added noise. For an input signal fluctuating between 3 values (amplitude distribution with 3 peaks), the amplitude distribution extracted at low noise levels was an acceptable solution at high noise levels (X^2, $p > 0.9$), but not a unique one. When non-linear interaction was assumed between signal and noise, the signal extracted under low noise conditions failed to meet criterion under high noise conditions ($p < 0.01$). In similar analysis of Ia-EPSPs we found that the distribution extracted under low noise was not an acceptable solution under high noise conditions suggesting non-linear interaction between background synaptic noise and Ia-EPSPs. Simulations in which noise was added linearly to the EPSPs (via computer techniques described above) to achieve baseline variance characteristic of the physiological high noise condition confirmed that the low noise solution (i.e. EPSP amplitude distribution) could apply during high noise conditions. These observations suggest that non-linear interactions may occur between spontaneous and Ia-evoked EPSPs particularly under conditions of increased baseline synaptic noise. This requires consideration when analyzing fluctuations of Ia-EPSPs, particularly when relating fluctuations to the number of active synaptic release sites. Partly supported by grants NIH NS 09196, NS 16996 and CONACyT PCCBBNA 021782.

469.18 SINGLE FIBER GROUP Ia EPSPs PRODUCED SIMULTANEOUSLY IN PAIRS OF MOTONEURONS: STUDIES OF CORRELATED ACTIVITY IN THE MOTONEURON POOL. P. Rudomin, M. Solodkin*, I. Jimenez*, & L. M. Mendell. CINVESTAV, Mexico and SUNY-Stony Brook, N.Y. 11794

The individual EPSPs produced in simultaneously impaled pairs of motoneurons (n=9) were studied in Nembutal anesthetized cats using spike triggered averaging techniques. In all but one motoneuron pair there was no increase in the coefficient of correlation above baseline levels at the time of the EPSPs. This could imply that the Ia EPSP fluctuations were not correlated, or else, that uncorrelated background synaptic noise precluded detection of correlated Ia EPSP fluctuations. Further analysis was carried out averaging both EPSPs over all trials to permit cursor positions for EPSP onset and peak to be determined. The amplitude of each EPSP produced in single trials in one (reference) motoneuron was measured using the predetermined cursor positions and assigned into one of several groups according to amplitude. The mean EPSP produced in the other (test) motoneuron was determined for trials in which EPSP in the reference was in each range of amplitude. In some cases the largest mean EPSP in the test occurred in the trials associated with the largest EPSPs in the reference, and smallest EPSPs in both also occurred in the same trials, etc. In other pairs of motoneurons there were no changes of EPSP amplitude in the test in association with differences in EPSP amplitude in the reference, indicating no correlated fluctuations of EPSPs in the motoneurons. In cases where parallel changes were observed we tested whether the correlated fluctuations were due to parallel changes in synaptic noise that would add to the Ia EPSP under study, thereby resulting in apparently correlated EPSP amplitudes in the motoneuron pair. This evaluation was carried out by moving the cursors in advance of the EPSPs to measure the amplitude of the baseline synaptic noise in single sweeps. In some pairs it was found that the baseline synaptic noise covaried in the reference and test motoneuron with a slope (Δ EPSP test/Δ EPSP ref) similar to that obtained by plotting the grouped mean Ia EPSP amplitudes of the test versus the reference motoneurons. In such cases we must conclude that most of the apparent correlated fluctuations in Ia fiber EPSPs results from correlation in additive baseline noise. However, in other cases correlated fluctuations of Ia EPSP motoneuron pairs were substantially different than those of the noise suggesting that the Ia EPSPs themselves were exhibiting correlated fluctuations. Taken together these data indicate that activity in homonymous motoneurons tends to be correlated but that under the present experimental conditions other factors besides intrinsic Ia EPSP fluctuations may predominate in determining these correlations. Partly supported by grants NIH NS09196, NS 16996 and CONACyT PCCBBNA 021782.

469.19 Morphology of primary afferent fibers and ventral horn cells in organotypic cultures of rat spinal cord. U. Braschler*, E. Kuenzi*, H. Oetliker* and H.-R. Lüscher. Department of Physiology, University of Berne, CH-3012 Berne, Switzerland.

Transverse slices (350 µm) of fetal rat (ED14) spinal cord with attached dorsal root ganglia (DRG) were cocultured with small pieces of skeletal muscle obtained from new born rats, using the modified roller tube technique of Gähwiler (J. Neurosci. Meth. 4: 1981). During the first two weeks in culture the spinal cord flattened into a relatively thin sheet preserving many morphological characteristics of spinal cord. The DRGs, connected to the spinal cord by fiber bundles, migrated away and flattened into a monolayer of neurons. Neurites from the ventral part of the spinal cord grew out into muscle fibers which differentiated from satellite cells in the muscle explant. Spontaneous contractions of myocytes could be observed as early as four days after explantation. Cross striation could be seen after thirteen days.

Local application of very small cristals of HRP into the muscle explant stained groups of relatively large multipolar neurons in the ventral part of the spinal cord. Their axons could be followed into the muscle explant. The same type of neurons was positively stained with acetylcholinesterase. The mean cell body diameter of these neurons was 41 µm (range: 20-67 µm, n = 20) and the mean number of stemdendrites observed was 4.2 (range: 3-7, n = 20). The mean dendritic length was 390 µm (range: 240- 620 µm, n = 10). The branching pattern of the dendritic apparatus was rather simple. These neurons are most likely motoneurons which functionally innervate the skeletal muscle explant.

Intracellularly stained (HRP and Lucifer Yellow) DRG-cells featured a great variety of intraspinal trajectories. The central branches of the DRG-cells formed terminal arborizations in the dorsal part of the spinal cord explant. Some collaterals descended into the ventral region of the spinal cord explant, where they again formed relatively complex arborizations. We have not yet demonstrated the direct synaptic contact of these afferent fibers on to the presumed motoneurons, but electrical stimulation of a DRG resulted in contraction of the cocultured muscle fibers, suggesting the functional integrity of the mono- synaptic reflex arch.

The organotypic structure of the spinal cord culture with some of its input and output systems preserved together with the good accessibility to microelectrode exploration offers an attractive model to study the structural and functional interrelations of central and peripheral synapses, as well as the regeneration and function of skeletal muscle. (Supported by the Swiss National Science Foundation No. 3.265-0.85)

470.1 COMPARISON OF NEURAL DAMAGE INDUCED BY ELECTRICAL STIMULATION WITH FARADAIC AND CAPACITOR ELECTRODES. D.B. McCreery, W.F. Agnew, T.G. Yuen, and L.A. Bullara. Huntington Medical Research Institutes, Neurological Research Laboratory, 734 Fairmount, Pasadena, CA 91105.

Arrays of platinum (faradaic) and anodized, sintered tantalum pentoxide (capacitor) electrodes were implanted bilaterally in the the subdural space of the parietal cortex of the cat. Two weeks after implantation both types of electrodes were pulsed with identical waveforms consisting of charge balanced, symmetric, anodic-first pulse pair, 400 μsec/ph, with charge/ph of 800-1000 μC, 80-100 μC/cm² for 7 hours. Tissues beneath both types of electrodes were damaged but damage was slightly greater beneath platinum electrodes. At the ultrastructural level, in animals killed immediately after stimulation, hyperchromic and shrunken neurons were intermixed with neurons showing early intracellular edema. Glial cells appeared essentially normal. In animals killed 1 week after stimulation most of the damaged neurons had recovered but the presence of shrunken, vaculated and degenerating neurons showed that some of the cells were damaged irreversibly. We conclude that most of the neural damage from stimulations of the brain surface derives from processes associated with passage of the stimulus current through tissue rather than electrochemical reactions associated with current injection across the electrode-tissue interface, since such reactions only occur with the faradaic electrodes (Supported by Contract #NO1-NS-62397, Fundamental Neuroscience Program, National Institutes of Health).

470.2 Reversal of Increased Muscle Fatigue in Paraplegic Patients by Electrical Stimulation. G. Vrbova*, M.M. Dimitrijevic*, M. Partridge*, J. Halter, Leo Verhagen Metman* Section of Restorative Neurology and Clinical Neurophysiology, Department of Rehabilitation, Baylor Coll. Med., Houston, TX, 77030.

It is now well established that activity is an important factor in determining the properties of skeletal muscle fibers such as their ability to withstand fatigue. Increased activity induced by chronic electrical stimulation reduces fatigability in animal experiments (Pette and Vrbova, Muscle and Nerve, Vol. 8, pp. 6-9, 1985) as well as in human muscle (Scott, Vrbova, Hyde and Dubowitz, J. Neurol Neurosurg Psychiat. 48: 774-787, 1985). In view of this we tested whether the muscles of patients that have been inactive due to upper motor neuron paralysis for long periods of time would become fatigable, and whether the increased fatigability could be reversed by chronic electrical stimulation.

The fatigability of the muscle was assessed by recording the decrease of force developed in response to repeated stimulation of the tibialis anterior muscle at its motor point by means of a train of stimuli at 40 Hz for 250 milliseconds of each second for three minutes. In control subjects the force of the tibialis anterior decreased to 70% ± 12% of its original force, while in paraplegic patients examined six months or later after paralysis, the force decreased to 35% ± 40%. In a group of paraplegic patients with no voluntary control of both legs the muscles of both legs were stimulated for three to four hours a day. Three months later, when their fatigability was again assessed, their muscles had become significantly more fatigues resistant. Thus electrical stimulation can replace voluntary activity in maintaining fatigue resistance of paralyzed muscles.

470.3 Simulation of Feedback Control of Joint Position and Stiffness by Electrical Stimulation of Antagonist Muscles. P.E. Crago, W.R. Fisher*, R.R. Carter* and L.K. Liu*, Depts. of Biomed. Eng. and Orthopaedics, Case Western Reserve University, Cleveland, OH 44106.

Neuroprostheses have been developed for restoration of hand grasp in quadriplegics and posture and gait in paraplegics. The design of feedback control systems for these applications has also been investigated, but the design has been carried out heuristically due to a lack of adequate dynamic models describing the control of stimulated muscle and the biomechanics. We have implemented a computer simulation of the feedback control of a single joint by stimulation of antagonist muscles. Feedback control of joint position and stiffness are being investigated.

A flexible simulation program was implemented. The following elements define the system: a flexor and an extensor muscle; separate internal and external loads; a feedback controller; and a map relating the controller output to the stimuli applied to the two muscles. Each muscle is modeled independently. Muscle force is calculated as the product of the isometric force produced by the stimulation and two terms describing the force-velocity and length-tension properties respectively. The internal load is modeled to represent the passive torque-angle properties at the joint. The external load represents mechanical objects in the environment that make contact with the limb. Both loads are modeled as general second order linear systems. A discrete-time control system was implemented, with a choice of feedback parameters (force, position or both for stiffness regulation)and a schoice of order. Force feedback is derived from the contact force between the limb and the external load. The modulation of the stimuli applied to each muscle is based on a method employed previously; the output of the controller is mapped to the values of the stimuli to be applied to each muscle. With this scheme, the degree of coactivation of the muscles can be varied in a preprogrammed manner. Mathematically, the simulation is broken down into two parts: the controller calculations and the stimulation of the muscle are carried out at discrete times corresponding to the stimulus period; between each stimulus, the muscle and load equations are integrated using a variable step-size Runge-Kutta method.

The simulation is very general and almost all parameters can be altered freely. Two specific questions are being addressed. The first is the performance of a control system under different constant external loads and the second is the performance at load transitions such as take place when reaching for and grasping an object.

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470.4 SELECTIVE ACTIVATION OF SMALL MOTOR AXONS BY SPIRAL CUFF ELECTRODE AND QUASI-TRAPEZOIDAL STIMULUS PULSES. Z.P. Fang* and J.T. Mortimer. Applied Neural Control Lab., Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106

We have found a method to activate electrically small fibers without activating large fibers in the same nerve trunk. The method utilizes a previously developed technique for generating unidirectionally-propagating action potentials (van den Honert, C., et al, Science, 206:1311, 1979). Quasi-trapezoidal stimulus pulses were delivered by a tripolar cuff electrode to excite all nerve fibers of different diameters under the center-positioned cathode while blocking selectively large fibers under the side-positioned anodes. The quasi-trapezoidal shaped current pulses, with a plateau of 350 uS and an exponential decay, insured the blockage of propagating action potentials and prevented the anodal break effect. The tripolar configuration of the electrode restricted current inside the cuff and prevented the virtual cathode effect. The self-curling spiral structure made the cuff electrode easy to install and reduced the likelyhood of mechanical trauma to the nerve (Naples, G.G., et al, Soc. Neurosci. Abstr., 12:1307, 1986).

Acute experiments were performed on 13 adult cats. The branch of the sciatic nerve innervating the medial gastrocnemius was surgically isolated for a length of 3-4 cm to install the spiral cuff electrode. After laminectomy, action potentials were recorded from L7 ventral roots. As the stimulus pulse amplitude increased from zero to about 0.1 mA, the compound action potential amplitude increased rapidly to the maximum with several peak representing alpha and gamma fiber groups of different conduction velocities. When the stimulus current was raised beyond 1 mA, the amplitude of the compound potential started to decrease, with the early peaks in the alpha complex being suppressed first, sparing the slower ones. At even higher current amplitude of about 3 mA, the whole alpha complex faded, leaving only the gamma components. After compound action potential recording, the L7 ventral root was teased down until a small fascicle containing only two to three alpha fibers of different conduction velocities was obtained. The recordings showed that the faster components of alpha waveform could always be suppressed while sparing conduction of the slower ones.

This method has been used to activate selectively small motor units in skeletal muscles in an attempt to obtain a slow-to-fast twitch recruitment order in electrically activated muscles. It may also be used for selective activation of small sensory fibers in peripheral nerves. In comparison with DC block, high frequency block, and triangular voltage pulse block with hook electrodes, this method has the merits of not causing asynchronous firing, less charge injection and easy to implement in chronic application.

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470.5 Analysis of Tendon Transfer Surgery in the Tetraplegic Upper Extremity. L.S. Mendelson*, P.H. Peckham*, M.W. Keith, A.A. Freehafer*. Depts. of Biomed. Eng. and Orthopaedics, Case Western Reserve University, Cleveland, OH 44106.

Restoration of voluntary hand grasp and elbow extension in the tetraplegic upper extremity has been achieved with tendon transfers. These procedures involve transfer of a voluntary motor into the insertion of a paralyzed muscle. Two patient groups were evaluated. The first had weak C6 function, with only the ECRB, ECRL, brachioradialis (BR), biceps and brachialis intact distal to the shoulder. The BR was transferred to the FDP to provide finger flexion, the ECRL (via a graft from the FDSr) to the AbPB to provide thumb opposition, and the posterior head of the deltoid to the triceps. The second group had strong C6 function, with voluntary control of the PT, FCR, and triceps muscles in addition to those listed above. These subjects had the BR as the motor for finger flexion, and the PT for thumb opposition.

Three patients in each group were evaluated at least one year after surgery. Thumb and finger forces and muscle firing patterns were measured with external transducers and electromyography.

Significant differences in thumb pinch and finger grasp strengths exist between the two subject populations. With the elbow 135° extended and the wrist neutral, the average pinch strengths were 10.6 (s.d.=2.9) N for the weak and 26.4 (s.d.=7.4) N for the strong subjects. The average grasp strengths were 13.0 (s.d.=2.1) N and 23.3 (s.d.=10.3) N. One cause of the difference in thumb force is likely related to the use of different motors. The PT has a greater tension fraction (5.5 %) than the ECRL (3.5 %), and thus is expected to exert more force (Brand, P.W., Hand Surgery, 6(3):209, 1981). The differences in finger flexion force may be caused by elbow joint stabilization. Subjects with voluntary triceps were better able to stabilize the elbow thus producing more finger force. All subjects showed change in pinch and grasp forces when muscle length was changed by varying the wrist and elbow positions.

New phasic patterns of muscle activity were observed in the transferred muscles. All motors contracted actively to produce motion and force in the digits. Each subject cocontracted an antagonist to elbow flexion (triceps or posterior deltoid) during activation of the brachioradialis. The weak C6 subjects generally contracted all the voluntary muscles in the forearm during attempted grasp and pinch. The strong C6 subjects had a greater ability to isolate the firing of a transferred motor from synergistic muscles and from other transferred muscles.

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470.6 REACTION AND MOVEMENT TIMES IN ELDERLY SUBJECTS AND PATIENTS WITH PARKINSON'S DISEASE. J.D. Brown and J.D. Cooke, Dept. of Clinical Neurological Science and Dept. of Physiology, University of Western Ontario, London, Ontario, Canada.

Difficulty in initiating movements and slowness of movement are commonly observed sequellae of Parkinson's disease. In the extreme these may result in the complete absence of movement (akinesia) in these patients. This akinesia may, however, be overcome in some situations if an appropriate sensory cue is given to the patient for movement initiation. We have studied the effect of different sensory cues on reaction and movement times in Parkinsonian patients. Since motor ability changes with age, comparative studies were performed on age and sex matched normal subjects.

Subjects performed 40 deg flexion/extension movements about the elbow between two fixed targets. Subjects were instructed to initiate movement as quickly as possible upon cue presentation and to move to the new target "as quickly and as accurately" as possible. Visual, auditory, kinesthetic (limb perturbation) and vibratory (vibration applied to the forearm) cues were given. The interval between successive cue presentation varied randomly from 3-6 sec. A total of 12 patients (treated and untreated) and 12 age and sex matched controls were studied.

Overall, there was little difference in reaction times between the patients and the control subjects. Reaction times did not vary consistently with the type of sensory cue in either group. Total movement time and the time from movement initiation to peak velocity were generally greater in the patients. However, different subjects often employed different strategies for making the movements. Some subjects overshot the desired target and made a rapid retury movement into the target. Others slowed well before the target was reached, entering the target with a slow, prolonged corrective movement. The former subjects generally had shorter movement times and times to peak velocity than the latter.

The data suggests that some of the changes in motor ability seen in the elderly parallel those seen in patients with Parkinson's disease. The data also suggests that movement initiation and performance in Parkinsonian patients is not dependent on the nature of simple cues for movement. Improvement in akinesia which has been reported upon presentation of specific cues is probably related to the pattern of the cues persented rather than to the specific sensory modality of the cue.

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470.7 VOLUNTARY OROFACIAL MOTOR CONTROL IN SCHIZOPHRENIA AND TARDIVE DYSKINESIA MP Caligiuri, MJ Harris *, T Murry * and DV Jeste
VA Medical Center, San Diego, CA. 92161.

Orofacial dyskinesia is present in over 80% (Jeste and Wyatt, 1982) of patients with tardive or spontaneous dyskinesia. Yet, instrumental approaches have generally not been applied to the assessment of motor control in schizophrenia or in patients with TD. The present paper reports the results of an investigation using strain gauge instrumentation to quantify voluntary control of labial, mandibular and lingual movements in schizophrenic patients with and without TD. The aims of the study were threefold: (1) to determine if voluntary orofacial motor control is disrupted in schizophrenia; (2) to determine if a relationship exists between TD and orofacial motor control; and (3) to identify subsystem impairments which contribute to the understanding of the pathophysiology of TD.

Methods Twenty schizophrenic(SZ) and twenty normal control(NC) subjects were studied. All SZ patients were medicated at the time of testing and had been on stable doses of neuroleptics for a period of at least two weeks. Of the SZ patients ten had moderate to severe TD. Movements of the lower lip, tongue and jaw were transduced using strain-gauge instrumentation. Analyses were made of performance on static and dynamic tracking tasks to evaluate group differences. The relationship between clinical ratings of TD (Abnormal Involuntary Movement Scale: AIMS) and the degree of fine motor dyscontrol was also tested.

Results Test-retest analyses for NC, SZ and TD subjects indicated high reliability of this instrumental approach as a means of quantifying orofacial motor control. The results indicated significant group differences on orofacial motor control with schizophrenic patients exhibiting poorer performance on both dynamic and static trials compared with normal subjects. In addition, TD patients exhibited more difficulty on both dynamic and static tasks compared with non-TD patients. Significant correlations were found between AIMS scores and dynamic motor control for lip (r=0.72) and tongue (r=0.60) but not jaw. There was no relationship between AIMS and orofacial static motor control. The results on differential subsystem impairments in TD suggested that labial control was more disrupted than lingual and mandibular motor control in that order.

Conclusions These findings suggest that fine orofacial position control may be disrupted in schizophrenia. The extent to which fine control of lip and tongue movements are compromised appears related to the severity of TD. Differential impairments within the orofacial system may contribute to a better understanding of the motor pathophysiology of TD. Studies on larger samples are underway.

470.8 **GAPS IN EMG ACTIVITY DURING ISOMETRIC WRIST FLEXION AND EXTENSION BY CHRONIC CVA PATIENTS.** S.S. Fitts, M.C. Hammond*, P.B. Nutter* and G.H. Kraft*. Rehabilitation Medicine, University of Washington, Seattle, WA 98195.

"Ratchety" motor unit recruitment is commonly recognized during clinical assessment of upper motor neuron paresis using needle EMG, yet the phenomenon has not previously been quantified. We analyzed gaps or silent periods 100 msec or longer in EMG activity recorded via monopolar needles from flexor carpi radialis(FCR) and extensor carpi radialis longus (ECR).

Six males and three females 49-77 ($\bar{X}$=62) years old had hemiparesis resulting from unilateral stroke 14-58 ($\bar{X}$=32) months previously. All patients and five healthy age/sex matched controls were right-hand dominant. Subjects were instructed to maintain maximal voluntary contractions against an isometric restraint apparatus which stabilized the wrist in a neutral position with the elbow at an angle of 135°. Trial durations of 3, 6, or 9 seconds were signalled by buzzers in a balanced random order.

Motor unit recruitment had more gaps in paretic than in control muscles ($p<.01$). In paretic muscles, the number of gaps increased with longer trial durations. Total active time was computed by subtracting the sum of all gap durations from the time between onset and offset of EMG activity. Since paretic muscles had less total active time than control muscles ($p<.01$), and paretic ECR had less active time than paretic FCR ($p<.05$), we examined the number of gaps per second of EMG activity. The number of gaps per second active was greater in paretic than in control forearms ($p<.01$). In paretic forearms, the number of gaps per second active was greater at longer trial durations ($p<.01$).

The greater number of gaps in paretic than control EMG activity may reflect a reduced number of active motor units within the receptive field of the electrode and/or the reduced firing rate and irregular firing pattern in single unit recordings reported by others. This quantification of "ratchety" recruitment is sensitive enough to distinguish the greater impairment of a wrist extensor than a wrist flexor muscle, and should prove useful for future comparisons of the severity of paresis and the progress of recovery.

NIDRR grant #G008435053 supported this research.

470.9 ISOMETRIC TREMOR AND MOST RAPID VOLUNTARY CONTRACTIONS IN PATIENTS WITH HEREDITARY MOTOR AND SENSORY NEUROPATHY (HMSN). E. Logigian*, K. Reiners*, H. Hefter* and H-J. Freund* (Spon: R. Young). Dept. of Neurology, University of Dusseldorf, D-4000, Dusseldorf 1, FRG.

In patients with HMSN types I (N=5) and II (N=5), we investigated isometric force tremor of index finger abduction recorded in steps from 0 to 70% maximal voluntary force (MVF), while simultaneously recording single motor units (SMU) from the first dorsal interosseous muscle (FDI). Peak tremor and SMU firing frequencies were then determined. In addition, we isometrically recorded most rapid voluntary contractions of index finger abduction over a range from minimal to maximal peak force, while simultaneously measuring rectified EMG from FDI. For each patient, the peak force (PF) of each contraction was plotted vs. its contraction time (CT) to obtain the slope (CT/PF) of this linear relation.

Peak tremor frequencies were significantly higher in HMSN II patients than in those with HMSN I matched for MVF of index finger abduction. In those who underwent needle examination, SMU and tremor peak frequency were positively correlated. Similarly, CT/PF was significantly lower in HMSN II than in matched HMSN I patients, but there was no significant difference between the two groups in CT of index finger abduction evoked by supramaximal ulnar nerve stimulation. In both groups, CT/PF and FDI EMG burst length were negatively correlated with MVF of index finger abduction.

Higher tonic and maximal SMU firing rates may underlie higher peak tremor frequencies and shorter voluntary contraction times, respectively in HMSN II compared to matched HMSN I patients. During most rapid voluntary isometric contraction, weaker patients with HMSN of either type employ longer agonist EMG burst length (and CT), than do stronger patients, to reach a given peak force.

470.10 VISCOUS LOADING IMPROVES MANUAL TRACKING ACCURACY IN HUMANS WITH CEREBELLAR INCOORDINATION. B.L. Morrice*, R.G. Lee, W.J. Becker, D.G. White*, and J.A. Hoffer. Dept. of Clinical Neurosciences, University of Calgary, Alberta, Canada T2N 4N1

When patients with cerebellar incoordination attempt to perform a relatively slow tracking task they often generate inappropriately high velocity movements. This study was undertaken to determine whether tracking performance could be improved by modifying the mechanical properties of a manipulandum used for manual tracking of a visual target.

Four patients with unilateral cerebellar lesions and four age-matched control subjects were presented with a visual display of a target moving horizontally across a screen in an unpredictable pattern at velocities of 0-30 degrees/sec. They were required to track the target by flexing and extending the wrist. After a learning period to familiarize the subjects with the apparatus, the viscosity, stiffness, and inertia of the apparatus were systematically altered. Viscous resistance and stiffness were provided by feeding back the velocity and position, respectively, to the amplifier that powered a torque motor. Inertia was altered by adding weights to the manipulandum. The cumulative difference between the signals representing wrist position and target position provided a measure of error for each 20 second trial. The amount of EMG activity in flexors and extensors was also measured, and the degree of coactivation estimated. Signals representing target and wrist position were differentiated to examine movement velocity.

When patients performed the tracking task with the affected arm, error scores were significantly higher ($p<.005$) than in the clinically unaffected arm. Increasing viscosity improved tracking accuracy in the affected arms of patients ($p<.010$), but not in the clinically unaffected arms nor in control subjects. Increasing the stiffness produced no consistent change in error scores. Addition of inertia either made no significant change in the accuracy, or increased the error.

These results suggest that application of a viscous interface may dampen unwanted movements and improve accuracy of performance in patients with cerebellar incoordination.

Supported by the Alberta Heritage Foundation for Medical Research and the MRC (Canada).

470.11 COMPARING TARGETED FEEDBACK AND MOTOR COPY TRAINING PROCEDURES IN THE RESTORATION OF UPPER EXTREMITY FUNCTION AMONG CHRONIC NEUROLOGICAL PATIENTS. S.L. Wolf* and D.I. Edwards. Dept. of Rehab. Med., Emory Univ. Sch. of Med., Atlanta, GA 30322.

This study sought to determine if targeted (conventional) electromyographic (EMG) feedback training (N=13) was superior to a novel task of motor (efference) copy (N=13) in producing functional and neuromuscular changes among chronic stroke and head injured patients. Targeted training consisted of dual channel monitoring of specific muscle groups in the hemiplegic limb, while motor copy required controlled activity of bilateral homologous muscle groups for which a visual representation of a muscle signal from the uninvolved muscle served as a template to be matched from output of the involved muscle. Thirty treatments followed specific protocols with functional and neuromuscular evaluations given as 5 baseline sessions prior to treatment, after every 10 treatment sessions and at 3 and 6 month follow ups. Both groups showed significant improvements in time and force measures of most of the 12 functional tasks with the target muscle training group showing more significant changes among tasks overtime (Friedman 2 way ANOVA). Between group analyses (Mann Whitney) indicates that among 7 tasks the target trained group showed more significant changes than motor copy.

Both groups demonstrated significant increases in active shoulder flexion range of motion whereas the targeted training group also showed significant increases in active wrist flexion and finger extension. The targeted training group also demonstrated a significant increase in mean EMG activity of anterior deltoid, biceps and wrist extensor musculature. These changes in neuromuscular characteristics may account for the greater number of significant functional improvements on the targeted training group.

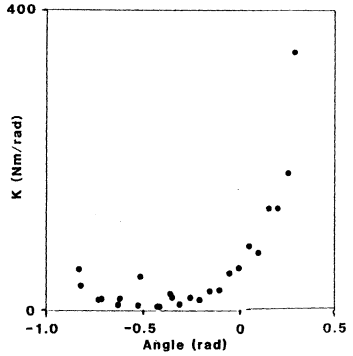
(Supported by the National Institute for Disability and Rehabilitation Research (Grant # G008300041))

470.12 PARADIGM FOR THE CLINICAL ASSESSMENT OF QUANTITATIVE JOINT FUNCTION. P.L. Weiss, R.E. Kearney, I.W. Hunter, R. Morier* and E.Dannenbaum*. School of Physical and Occupational Therapy and Biomedical Engineering Unit, McGill University, Montreal, Canada, H3G 1Y5.

Although it is well recognized that neuromuscular disease can severely disrupt functional ability, objective, valid and reliable measures of abnormal muscle and joint behaviour are almost completely lacking. Previous work in this laboratory has resulted in the development of a suite of experimental and analytical techniques, based on system identification, for the rapid, quantitative measurement of the mechanical and reflex properties of the human ankle. The objective of this presentation is to describe a paradigm for the clinical assessment of normal and abnormal quantitative joint function and to report some of our preliminary results.

The subject's ankle is slowly driven (0.05 rad/s) by an electrohydraulic actuator through a trajectory covering most of its range of motion while small amplitude (0.05 rad) random perturbation of angular position are imposed. The joint position, torque and surface EMGs from the ankle muscles are recorded; these experimental records are divided into short (2.5 s) records within which only small changes of mean joint angle occur. Each segment is analyzed separately to yield a series of dynamic descriptions of the passive ankle mechanics.

The results from one subject are shown in the figure where passive ankle stiffness is plotted as a function of ankle angle. The typical passive stiffness/position profile (highly compliant joint at mid-range positions that becomes considerably stiffer at maximum dorsiflexion) is evident.



470PO REVERSIBLE CONDUCTION DISORDER IN RAT TIBIAL NERVE FROM REPETITIVE ELECTRICAL STIMULATION. A.Y. Liu*, C-Z. Hong and J. Yu. Department of Physical Medicine and Rehabilitation, University of California, Irvine Medical Center, Orange, CA 92668.

Repetitive electrical stimulation (100 mA, 0.05 msec, 20 Hz) was applied on one side of the tibial nerves of 16 adult Sprague-Dawley albino rats between the knee and the ankle for 10 min each session, 3 times per week. Nerve conduction velocity (NCV) across the stimulated segment, distal latency from ankle stimulation, the amplitude and duration of the evoked compound muscle action potential (CMAP) were measured from bilateral tibial nerves twice per week. The NCV and the amplitude of CMAP from proximal stimulation were significantly reduced on the side of stimulation as compared to the control side by one week after the beginning of stimulation. The distal latency and the duration of CMAP remained unchanged until 4 weeks after stimulation. At that time, both the distal latency and the duration of CMAP were increased (with evidence of temporal dispersion of CMAP). Another study with 10 Hz, instead of 20 Hz, revealed that the only change observed was a reduced amplitude of CMAP from proximal stimulation. All changes returned to normal within one week after cessation of stimulation. It is concluded that repetitive electrical stimulation with high intensity may adversely affect nerve conduction. Clinical application of electrotherapy should be applied cautiously.

OPIATES, ENDORPHINS AND ENKEPHALINS: ANATOMY AND CHEMISTRY III

471.1 THE ANATOMICAL DISTRIBUTION OF PEPTIDES SHOWING PRODYNORPHIN IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF THE GOLDEN HAMSTER (Mesocricetus auratus). C. Neal, Jr.* and S. W. Newman (SPON S. Hicks). Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109

The anatomical localization of the prodynorphin opioid precursor system has been studied in the rat in detail (Fallon, J.H., JCN, 249:293-336). This system has several of the active opioid sequences present within the original precursor molecule prior to processing. Polyclonal antibodies to dynorphin B (1-13), dynorphin A (1-8) and C-peptide were used in an immunocytochemical study to generate a prodynorphin map of the Golden hamster in coronal and parasaggital brain sections. The C-peptide antibody is directed at the C-terminus of dynorphin B (1-29) and, therefore, is lacking cross-reactivity with the leu-enkephalin amino acid sequence found in the dynorphin opioid peptides. Areas of heaviest prodynorphin cell staining include the anterior continuation of the hippocampus, lateral septum, dentate gyrus, Ammon's horn (CA1-4), bed nucleus of the stria terminalis (BNST), medial nucleus of the amygdala (AMe), central nucleus of the amygdala, medial preoptic area (MPOA), supraoptic nucleus, retrochiasmatic supraoptic nucleus, ventromedial nucleus of the hypothalamus, paraventricular nucleus of the hypothalamus, lateral hypothalamus, reticular formation, peripedunucular nucleus, nucleus ambiguus, accessory motor nucleus of the fifth nerve, and the solitary nucleus. Heavy fiber and terminal staining is observed in many of the above areas as well as in the ventral pallidum dentate gyrus, arcuate nucleus of the hypothalamus, periventricular nucleus of the thalamus, paratenial nucleus of the thalamus, parabrachial nucleus, substantia nigra (SNR), medial habenula and nucleus of the solitary tract.

Though the locations of these prodynorphin-containing neurons are very similar in general to staining generated in the rat, several species differences do appear to exist. In the globus pallidus, caudate-putamen and SNR, known areas of prodynorphin cell and/or fiber staining in the rat, weak staining is observed in the hamster. In contrast, in the MPOA, BNST, and AMe, areas that lightly stain for prodynorphin-containing fibers and cells in the rat, heavy cell and fiber staining is observed in the hamster. The staining variations may reflect important differences in the functional significance of MPOA, BNST, and AMe between the two species. In the hamster, but not in the rat, chemosensory pathways through the AMe & BNST to the MPOA are essential for normal male sexual behavior.
(supported by NIH:NS20269 to SWN)

471.2 COLOCALIZATION OF ENKEPHALIN AND CCK IN A PORTION OF THE SPINOTHALAMIC TRACT. J.C. Lauterborn*, P.S. Chang*, and C.M. Gall (SPON: D.D.Williams). Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Recent work in our laboratory has shown that the functionally antagonistic peptides enkephalin and cholecystokinin (CCK) are colocalized in discrete areas of the rat brain. In particular, we have observed double-labeled axons and puncta within two areas known to receive innervation from the spinothalamic tract: rostral intralaminar thalamic nuclei and a zone overlying the medial lemniscus and extending into the periaqueductal grey matter at the mesodiencephalic junction. The present study was conducted to determine if the enkephalin/CCK-containing fibers in these areas arise from the spinal cord.

Adult male Sprague-Dawley rats were used. In the first group, the spinal cord was either hemisected at C1 (n=4) or fully transected at a low thoracic level (n=3). Two weeks later, the rats were sacrificed by perfusion with 4% paraformaldehyde and the brains were processed for the immunocytochemical localization of enkephalin and CCK using the peroxidase antiperoxidase technique. Fibers and puncta containing both enkephalin immunoreactivity (ENK-I) and CCK immunoreactivity (CCK-I) were eliminated from the caudal, mesodiencephalic, system ipsilateral to the C1 hemisection. Following the full low thoracic transection, immunoreactive fibers were eliminated from this area bilaterally. In neither case was there a diminution of ENK-I or CCK-I in the intralaminar complex. These results suggest that cells below the thoracic level project to the mesodiencephalic field and that the ENK/CCK co-containing fibers of the intralaminar complex do not originate from the spinal cord.

A second group of rats (n=5) received bilateral injections of Fluoro-Gold into the area of the doubly immunoreactive fibers at the mesodiencephalic border. Fourteen days later, one day prior to sacrifice, 120 ug colchicine was injected into the dural sac at the caudal tip of the cord. Horizontal sections of lumbar/sacral spinal cord were processed with an indirect immunofluorescence technique for the simultaneous localization of ENK-I and CCK-I. In lumbar segments 4 and 5 we observed a population of cells, directly lateral to the central canal, which contained retrogradely-transported dye, ENK-I, and CCK-I. Other cells, dorsal to the central canal within lumbar segments 2 and 3, contained both ENK-I and CCK-I but no dye.

These data demonstrate that enkephalin and CCK are colocalized within a component of the spinothalamic tract which arises from cells in the intermediate grey of the lumbar cord and terminates at the mesodiencephalic junction. This suggests that some of the interactive influence of enkephalin and CCK over nociception occurs at spinothalamic synapses where these two peptides are colocalized and presumably coreleased. (Supported by BNS 8417098 and RCDA NS 00915 to C.G.).

471.3 DYNORPHIN A-IMMUNOREACTIVE NEURONS IN CA FIELDS OF RAT HIPPOCAMPUS ARE REVEALED BY 6-HYDROXYDOPAMINE. J.F. McGinty and J.R. Loesche*. Dept. of Anatomy, East Carolina University School of Medicine, Greenville, NC 27858.

Dynorphin immunoreactivity has been described by several groups to be located in the hippocampal formation exclusively in the dentate granule cell-mossy fiber system. In contrast, electrophysiologists have recorded substantial effects of exogenously applied dynorphin in the CA1 hippocampal field in vivo and in vitro. In normal rats perfused with a 4% paraformaldehyde solution with or without 15% saturated picric acid, only sparsely scattered, varicose fibers have been observed in CA1 by immunocytochemistry. However, one week after intraventricular (200 µg/10 µl) or intracerebral (8 µg/4 µl) injections of 6-hydroxydopamine (6-OHDA), numerous dynorphin A immunoreactive cell bodies were visualized in CA1-4, fewer in the dorsal subiculum, of hippocampus. The cell bodies are located principally in stratum radiatum (s.r.) and stratum oriens (s.o.); however, some somas are located close to or in the pyramidal cell layer. Neurons in s.r. are large and have multiple, long radial processes extending in an apical direction through s. lacunosum to the edge of (but not penetrating) stratum moleculare and in a basal direction to the pyramidal cell layer. Neurons in s.o. are often located deep and some are in the alveus; they have a multipolar or horizontal configuration with many processes extending parallel and/or perpendicular to the pyramidal cell layer. Fewer neurons were seen in CA3 and CA4 and none were seen in the dentate gyrus except for occasional immunopositive granule cells which have been described previously. These data raise the possibility that dynorphin metabolism in cell bodies in hippocampal CA fields may be tonically inhibited by norepinephrine. Electrophysiological recordings of CA1 pyramidal cell activity in the presence of different combinations of opioid and norepinephrine agonists and antagonists may yield some insight into the interactions between these two neuroactive substances in the hippocampal formation. Supported by DA 03982.

471.4 OPIOCORTIN AND CATECHOLAMINERGIC PROJECTIONS TO PERIAQUEDUCTAL GREY AND RAPHE NUCLEI. L. Sim*, E. Lynd* and S.A. Joseph. Neuroendocrine Unit, Univ. of Rochester, Rochester, NY 14642.

Anatomical and physiological evidence has implicated the periaqueductal grey (PAG) and certain raphe nuclei in opioid analgesia. Electrical stimulation or morphine microinjection into ventral and lateral PAG, a region rich in opiate receptors and opiocortin fibers, elicits a profound, naloxone-reversible analgesia. Recent studies implicate the nucleus tractus solitarius (NTS), an opiocortin/opiate receptor rich region, as a participant in endogenous opioid analgesic mechanisms. This study was undertaken to evaluate whether opiocortin projections to PAG and raphe nucleus emanate from arcuate or NTS opiocortin neurons. Wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) was selectively placed throughout the rostral-caudal extent of the PAG and neighboring raphe regions as well as medullary raphe nuclei. Forty-eight hours later rats were either perfused with Kunio's fixative or injected intraventricularly with colchicine. Colchicine treated animals were sacrificed after 24 hrs. WGA-HRP activity was demonstrated using routine immunocytochemistry intensified with nickel DAB rendering a black to purple reaction product. The same sections were then stained immunocytochemically for various opiocortin neuropeptides and neurotransmitters. The distribution of the cells containing reaction product were identified and mapped throughout brain.

Retrogradely labeled neurons were consistently observed in the arcuate and NTS regions following injections in PAG as well as the dorsal raphe nucleus (DR). Dual cell body labeling indicate opiocortin and catecholaminergic afferents to these regions. Noradrenergic perikarya in NTS containing HRP/WGA reaction product were also observed with injections in PAG and DR. Perikarya containing HRP/WGA reaction product were also observed in the zona incerta, ventromedial nucleus, lateral hypothalamus, and premamillary nucleus with PAG injections and in amygdala, supraoptic nucleus, hippocampus, lateral hypothalamus, habenula, substantia nigra and locus coeruleus with dorsal raphe injections.

These results confirm our previous finding that opiocortin neurons from arcuate alone project to PAG and both arcuate and NTS opiocortin neurons innervate raphe nuclei. (Supported by NIH grant NS21323 and Columbia Development Company.)

471.5 MET-ENKEPHALIN IMMUNOREACTIVITY IN DEVELOPING RAT CEREBELLUM: PRELIMINARY IMMUNOELECTRON MICROSCOPIC STUDIES. S.M. Prouty and I.S. Zagon. Dept. of Anatomy, The M.S. Hershey Med. Ctr. of The Pennsylvania State University, Hershey, PA 17033.

Endogenous opioid systems (endogenous opioids and opioid receptors) are known to regulate certain aspects of neurodevelopment. In a previous light microscopic study (Science 227:1049-1051, 1985), met-enkephalin immunoreactivity was localized in germinative cells (external germinal cells=EGL) of the developing rat cerebellum, but not in their adult neuronal counterparts (e.g., internal granule neurons=IGL). This observation extended previous reports in which opioids and receptors were only detected during the first 2 weeks of rat cerebellar development. In the present study, we addressed the question of the ultrastructural localization of endogenous opioids in developing and adult rat cerebellum using immunoelectron microscopy.

Sprague-Dawley rats, 11 and 40 days of age, were anesthetized and perfused intracardially with 4% paraformaldehyde, 0.25% glutaraldehyde in 0.07M Sorenson's phosphate buffer with sucrose (pH 7.4, 20°C) for 5 min. Brains were removed, postfixed in primary fixative for 3 hr, followed by postfixation in the primary fixative without glutaraldehyde for 12 hr. Vibratome sections (120 µm) of the cerebellum, in both sagittal and coronal planes, were stained with antisera to met-enkephalin (ImmunoNuclear), followed by goat-anti-rabbit IgG conjugated to peroxidase, and processed in diaminobenzidine. Sections were embedded in Epon.

Preliminary observations indicated the presence of met-enkephalin immunoreactivity in EGL cells of 11-day old animals. This activity was localized in the cytoplasm, and was not in the nucleus. It was associated with mitochondria, the plasma membrane, and aggregates in the cytoplasm. Immunoreactivity was not detected in parallel fibers of the molecular layer. Some immunoreactive product was found in the soma of IGL cells. Purkinje cells of 11-day old rats often displayed prominent staining in soma and dendrites, and was associated with organelles (except the nucleus) and dendritic spines. Control specimens showed no immunoreactivity. Observations of 40-day old rats revealed little staining in either Purkinje or IGL neurons.

These results confirm and extend earlier reports that endogenous opioids such as met-enkephalin play a specific role in cerebellar morphogenesis, and show that opioid activity is intracellular and associated with discrete cytological entities. Moreover, these observations are consistent with the thesis that endogenous opioids are natural trophic factors involved in cell proliferation and differentiation.

Supported by NIH grants NS-20500 and NS-20623.

471.6 CHARACTERIZATION OF OPIATE AND SIGMA RECEPTOR TYPES ON THE NCB-20 CELL MEMBRANE. L. Kushner* and R.S. Zukin. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

The neuroblastoma X Chinese hamster brain hybrid cell line NCB-20 is the only cell line known to contain benzomorphan-specific sites in addition to the delta opiate receptor (West, et al., 1982). In order to identify and characterize the receptor types present in this cell line, we have used highly specific radioligands. Binding of the delta-specific ligand [^{3}H]D-Pen^2,D-Pen^5-enkephalin (DPDPE) revealed a linear Scatchard plot with a binding affinity (Kd) of 7.7 nM and a binding site density (B_{max}) of 462 fmol/mg protein. This receptor density is very close to that determined for the universal opioid ligand [^{3}H]bremazocine (B_{max}= 465 fmol/mg protein). Specific binding could not be obtained using the mu-specific ligand [^{3}H]D-Ala^2,N-Phe^4,Gly-ol^5-enkephalin (DAGO). Binding of the K-specific opioid [^{3}H](-)ethylketocyclazocine (EKC) in the presence of saturating concentrations of mu and delta ligands was indistinguishable from background levels. Displacement studies revealed that both [^{3}H]bremazocine and [^{3}H](-)EKC can be totally displaced by D-Ala^2,D-Leu^5-enkephalin (DADLE) (IC_{50}=5 nM). The relatively kappa-specific ligand U50,488 displaces only at very high concentrations (IC_{50}=12 uM) and the specific kappa ligand U69,593 does not displace either radioligand. The binding site for the PCP/sigma-specific ligand [^{3}H]1-[1-(2-thienyl)cyclohexyl]-piperdine (TCP) had a K_d of 364 nM and a B_{max} of 8982 fmol/mg protein. The rank order of potency of a series of opiates, opioid peptides, and related drugs was determined to be TCP>U50,488>dexoxadrol>haloperidol> phencyclidine (PCP) > cyclazocine > levoxadrol > (+)SKF10,047> (-)SKF10,047 at this site, whereas DAGO, normorphine, DADLE, DPDPE, and EKC do not compete for binding. Binding of [^{3}H](+)SKF10,047 revealed only one class of sites with a binding affinity of 87 nM and a receptor density of 1703 fmol/mg protein. The rank order of potency of a series of opiates, opioid peptides and related drugs was determined to be (+)SKF10,047>haloperidol>TCP>U50,488>dexoxadrol>DPDPE at this site, whereas DAGO, normorphine and DADLE do not compete for binding. These two sigma-like receptors are, thus, distinct binding sites. The most notable distinctions between the NCB-20 sigma sites and those of rat and mouse are the binding affinities of TCP and (+)SKF10,047 and the binding of U50,488, a K-specific ligand, at those sites. Thus, NCB-20 cells have PCP/sigma-like and haloperidol/sigma-like receptors, in addition to delta opioid receptors, but are devoid of mu and kappa receptors. (Supported in part by NIH grant DA01843 and DA00069 (RSZ) and AM07513 (LK)).

471.7 LABELING OF THE HALOPERIDOL-SENSITIVE SIGMA RECEPTOR IN NCB20 HYBRID NEUROBLASTOMA CELLS WITH [3H]-(1,3)-DI-ORTHO-TOLYL-GUANIDINE AND IDENTIFICATION OF THE BINDING SUBUNIT BY PHOTOAFFINITY LABELING WITH [3H]-m-AZIDO-DI-ORTHO-TOLYL-GUANIDINE.

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[3H]-(1,3)-Di-ortho-tolyl-guanidine ([3H]DTG) selectively labels haloperidol-sensitive sigma sites in guinea pig brain membranes (Weber et al.,PNAS 83,pp.1-6,'86). Binding studies with [3H]DTG in NCB20 cell membranes were therefore conducted to further substantiate the identification of haloperidol-sensitive sigma sites previously reported for this line (Largent et al., Eur.J.Pharm.124, pp.183-87,'86). Scatchard plots of equilibrium saturation data revealed a single binding population of [3H]DTG binding sites with dissociation constant (Kd) and maximum binding values of 54nM and 6.4pmoles per mg. protein, respectively. Unlabeled DTG completely displaced binding of 0.9nM (+)[3H]3-PPP (a selective radioligand for sigma receptors previously analyzed in this cell line, Largent et al., Eur.J.Pharm. 124, pp.183-87,'86). Unlabeled (+)3-PPP, in turn, displaced binding of 0.9nM [3H]DTG completely. In drug competition studies, the drug potency profiles for (+)[3H]3-PPP and [3H]DTG binding to NCB20 cell membranes were different, inspite of their identical drug profiles in guinea pig brain membranes (Weber et al., PNAS 83,pp.1-6,'86). In general, the benzomorphan drugs and the butyrophenone, haloperidol, were less than one tenth as potent as inhibitors of [3H]DTG binding as compared to (+)[3H]3-PPP binding in NCB20 cell membranes. Studies are currently under way to determine the nature of the interaction between DTG and (+)3-PPP binding in these membranes.

Binding studies were also conducted with a photolabile derivative of DTG, [3H]-m-Azido-di-ortho-tolyl-guanidine ([3H]AzDTG). In the dark, this compound bound saturably, reversibly and with high affinity to NCB20 cell membranes and binding was completely inhibited by 10uM haloperidol. Photoaffinity labeling experiments were done using this ligand to obtain an initial size estimation of the binding subunit of the haloperidol-sensitive receptor in this cell line. This procedure led to the incorporation of radiolabel into a single band (M_r 29,000) as revealed by sodium dodecyl sulfate polyacrylimide gel electrophoresis followed by fluorography. This band corresponds to the same size as that identified in guinea pig brain membranes using the identical photoaffinity labelling protocol with [3H]AzDTG. Labeling of this band is completely blocked by 10uM haloperidol.

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471.8 THE SELECTIVE SIGMA LIGAND [^{3}H]-DTG BINDS TO SITES IN HUMAN AND GUINEA PIG CEREBELLA WITH DISTINCT PHARMACOLOGICAL PROFILES.

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SKF 10,047 (N-allylnormetazocine) is a benzomorphan opiate which causes hallucinations and dysphoria in humans and delirium in dogs. It has been postulated to be the prototypical ligand for the sigma opiate receptor. Biochemical studies on rodent brain membranes have revealed that the sigma receptor (the high affinity binding site for (+)[^{3}H]SKF 10,047) displays: 1)stereoselectivity for dextrorotatory isomers of the benzomorphans, in contrast to the (-)-preferring opiate receptors; 2)high affinity (5 nM) for haloperidol and moderate affinity for several phenothiazine and atypical neuroleptics, but essentially no affinity for dopamine or apomorphine. (+)[^{3}H]SKF 10,047 has also been recognized to crossreact at the high affinity PCP binding site [Sonders et al.(1987)TINS, in press].

We have undertaken a comparison of the drug selectivity profiles of the sigma binding sites in guinea pig whole brain and cerebellum and human cerebellum. We chose to study cerebellum since its regular cytoarchitecture makes it amenable to electrophysiological investigation and rodent cerebella contain a large preponderance of sigma compared to PCP binding sites. Binding assays were performed with the novel and selective sigma ligand [^{3}H]-DTG (1,3-di-o-tolylguanidine [Weber et al. (1986) PNAS 83: 8784] in 50 mM Tris/HCl, pH 7.4. Bound drug was separated from free by rapid filtration. Binding of 2 nM [^{3}H]-DTG was linear with tissue concentration between 200-1200 ug protein/ml. In guinea pig cerebellar membranes, scatchard analysis shows that DTG has an affinity (K_d 27±2 nM) and B_{max} (70±5 pmol/g wet weight) virtually identical to values reported for guinea pig whole brain (28 nM, 84 pmol/g). Likewise, the IC_{50} value of 22 drugs are nearly identical in cerebellar and whole brain membranes, displaying the hallmark selectivities mentioned above.

In contrast, human cerebellar membranes exhibit lower affinity and a lower number of binding sites (K_d 57±9 nM, B_{max} 41±5 pmol/g. Also striking is the absence of clear stereoselectivity for isomers of SKF 10,047, pentazocine and cyclazocine. The potencies of most drugs in displacing [^{3}H]-DTG from human cerebellar membranes are lower than those seen in guinea pig brains. Exceptional are fluphenazine, perphenazine, triflupromazine and chlorpromazine, whose IC_{50}'s are very nearly the same in the two tissues.

The results of further pharmacological characterization of [^{3}H]-DTG, (+)-3PPP, (+)[^{3}H]SKF 10,047 and [^{3}H]-TCP binding in human cerebellum will be presented.

This work is supported by NIMH grants MH4303 and MH42068 and by a grant from Synax, Inc.

471.9 PHYLOGENETIC DISTRIBUTION OF HALOPERIDOL-SENSITIVE SIGMA AND PHENCYCLIDINE BINDING SITES IN THE NERVOUS SYSTEM.

T.H. Yu, A.D. Weissman, and E.D. London. Neuropharmacology Lab., NIDA Addiction Research Center, Baltimore, MD 21224.

Benzomorphans and certain arylcycloalkylamines may mediate psychotomimetic effects through their interaction with specific receptors in the brain. These sites include sigma (σ) and phencyclidine (PCP) binding sites, which can be labelled with [^{3}H]haloperidol (HAL) and [^{3}H]N-(1-[2-thienyl]cyclohexyl)3,4-piperidine (TCP), respectively. Although σ and PCP sites differ in pharmacology and anatomical distribution, the possibility of a functional relationship between them has not been excluded. To gain insight into the origin and possible relation between σ and PCP sites, we performed a phylogenetic study of the distribution and affinities of the two sites. Specific binding to homogenates of neural tissue from representatives of thirteen animal classes was determined. Whole sea anemone and planaria, the ganglia and ventral nerve cords of earthworm and crayfish, and either regions or whole brains of cricket, hagfish, mustelus shark, goldfish, turtle, frog, chicken, guinea pig, and squirrel monkey were assayed. Saturation studies with HAL and TCP in brain homogenates of seven vertebrate species (shark, goldfish, turtle, frog, chicken, guinea pig, and monkey) were performed. Binding of HAL was determined in the presence of 75 nM spiperone, and nonspecific binding of HAL and TCP was defined using 100 μM d-SKF 10,047 and 100 μM PCP, respectively.

At a ligand concentration of 2.5 nM, significant TCP specific binding was found in all organisms examined, with the highest levels in the crayfish. Substantial HAL specific binding (at a ligand concentration of 1 nM) was found in all species except the hagfish and the two phylogenetically most primitive organisms examined, sea anemone and planaria, where only a small amount of binding was detected. In the hagfish, low levels of both TCP and HAL specific binding were observed. Scatchard analyses showed that HAL bound to brain homogenates of the vertebrate organisms examined with high affinity (Kd = 1 - 4 nM). Bmax was highest in frog brain homogenates, and decreased according to the following rank order: guinea pig, goldfish, turtle, shark, monkey, chicken. TCP also bound with relatively high affinity (Kd = 6 - 40 nM) to all of the homogenates, with the highest Bmax in the frog, followed in decreasing order by values in monkey, goldfish, guinea pig, turtle, chicken, and shark brains.

The present results indicate that both the σ and PCP binding sites are phylogenetically old and are distributed throughout the animal kingdom. Furthermore, the affinities of specific ligands for each of the binding sites appeared similar across several species of vertebrates.

471.10 INTERACTING LIGAND BINDING DOMAINS FOR NEUROLEPTICS AND OPIATE BENZOMORPHANS ON THE HALOPERIDOL-SENSITIVE SIGMA "OPIATE" RECEPTOR.

W.D. Bowen, S.B. Hellewell*, and K. McGarry*. Sect. of Biochem., Div. of Biology and Medicine, Brown Univ., Providence, RI 02912.

We have studied the interaction of haloperidol (HAL), N-allylnormetazocine ((+)-SKF 10,047)), (+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine ((+)-3-PPP) and 1,3-di-o-tolylguanidine (DTG) with the haloperidol-sensitive sigma receptor (HAL/sigma).

Irradiation of rat brain membranes with 254 nm UV light produced loss of binding of (+)-[^{3}H]3-PPP and [^{3}H]DTG. Irradiation for 10 min resulted in about 70% loss of binding for both ligands. Conversely, this treatment resulted in a 90% increase in (+)-[^{3}H]SKF 10,047 binding. The differential photosensitivity of binding of (+)-[^{3}H]3-PPP and [^{3}H]DTG compared to (+)-[^{3}H]SKF 10,047 indicates that their sites of interaction with the receptor macromolecule differ.

Scatchard analysis revealed binding of (+)-[^{3}H]3-PPP to a single class of sites with K_d = 90 nM and B_{max} = 14 fmole/mg protein. We investigated the mechanism by which HAL, DTG, and (+)-SKF 10,047 inhibited binding of (+)-[^{3}H]3-PPP by carrying out Scatchard analyses in presence of unlabeled inhibitor. HAL and DTG were competitive inhibitors, increasing the K_d while having no effect on B_{max}. By contrast, (+)-SKF 10,047 was an uncompetitive inhibitor, causing a decrease in both the K_d and B_{max}. These results suggest that HAL, DTG, and (+)-3-PPP interact with the receptor by competing for the same site. (+)-SKF 10,047 interacts via a different site. This observation is consistent with the differential photosensitivities of the binding sites for these ligands.

The effect of UV irradiation on interaction of ligand binding sites was assessed. Irradiation for 10 min caused a dramatic decrease in the ability of both (+)-3-PPP and haloperidol to inhibit binding of 5 nM (+)-[^{3}H]SKF 10,047. The IC_{50} of (+)-3-PPP and HAL increased 40-fold and 196,000-fold, respectively, upon irradiation. This decrease in potency is presumably due to destruction of the binding site for these ligands and occurs concurrently with the increase (+)-[^{3}H]SKF 10,047 binding.

These results are consistent with a structural model for HAL/sigma whereby the receptor is composed of two ligand binding domains. Neuroleptics such as haloperidol bind to a UV-sensitive domain. This domain also binds (+)-3-PPP and DTG. (+)-Benzomorphans such as (+)-SKF 10,047 bind to a UV-resistant domain. These domains are allosterically coupled such that binding of ligand to one domain affects binding to the other. This structural model may have important implications for the pharmacology of this receptor. (We thank Dr. E. Weber for his gift of [^{3}H]DTG.)

471.11 THE SIGMA-SELECTIVE PHOTOAFFINITY LIGAND [^{3}H]AZIDO-DITOLYLGUANIDINE SPECIFICALLY LABELS A 29 kD POLYPEPTIDE FROM GUINEA PIG BRAIN MEMBRANES M.P. Kavanaugh*, B. A. C. Tester*, J. F. W. Keana*§, and E. Weber, Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201 and §Department of Chemistry, University of Oregon, Eugene, OR 97403

The haloperidol-sensitive sigma receptor is a candidate for mediating the psychotomimetic effects of benzomorphans in humans. In addition to dextrorotatory benzomorphans, the receptor binds [^{3}H](+)3-[3-hydroxyphenyl]-N-(1-propyl)piperidine ([^{3}H](+)-3-PPP) and [^{3}H]1,3-di-o-tolylguanidine ([^{3}H]DTG) with high affinity. In order to elucidate its structure, photoaffinity labeling of the sigma receptor from guinea pig brain was accomplished using a novel radioactive photolabile derivative of DTG, [^{3}H]-m-azido-1,3-di-o-tolylguanidine ([^{3}H]AZ-DTG). In the dark, [^{3}H]AZ-DTG binds reversibly to sigma sites in brain membranes with high affinity (K=28 nM). The drug specificity profile of [^{3}H]AZ-DTG binding to brain membranes is identical to that of the prototypical sigma ligands [^{3}H]DTG and [^{3}H](+)-3-PPP. For photoaffinity labeling, membrane suspensions containing protease inhibitors were preincubated in the dark with [^{3}H]AZ-DTG, then filtered and washed over Whatman GF/B glass fiber filters. The filters were then irradiated with long-wavelength UV light for a 15 minute period. Filter-bound proteins were solubilized with 50 mM Tris pH 7.4, 0.1% sodium dodecyl sulfate. Solubilized proteins were subjected to SDS-polyacrylamide gel electrophoresis. Fluorography of the SDS-PAGE gels revealed that [^{3}H]AZ-DTG was selectively incorporated into a 29 kD polypeptide. Labeling of this polypeptide was completely blocked by the sigma ligands DTG, (+)-3-PPP, (+)pentazocine, and haloperidol at a concentration of 10μM, while labeling was unaffected by morphine, serotonin, dopamine, scopolamine, or GABA at the same concentration. These results represent the first estimate of the size of the binding subunit of the haloperidol-sensitive sigma receptor. Purification of this polypeptide is now under way.

This work was supported by NIMH grants MH40303 and MH42068 and by a grant from Synax, Inc., Cambridge, MA.

471.12 [^{127}I]β_h-ENDORPHIN BINDING: BIOCHEMICAL CHARACTERIZATION AND CROSS-LINKING STUDIES. J.A. Clark and G.W. Pasternak [SPON: W.F. Riker]. The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Cornell U. Medical College, New York, NY 10021.

Previously, our group has studied [^{3}H]β_h-endorphin binding in brain extensively. In these studies, we found that [^{3}H]β_h-endorphin labeled two classes of sites with high affinity (K_D < 1 nM). One corresponded to the mu_1 site while the other was selective for β-endorphin and did not appear to correspond to either morphine-selective mu (mu_2) or delta sites. In view of the extensive use of [^{127}I]β_h-endorphin to identify opioid binding sites through crosslinking approaches, we have examined its binding to brain membranes and examined its crosslinking. As with the tritiated material, [^{127}I]β_h-endorphin bound extensively to glass fiber filters and we therefore used a microfuge assay, although treating the filters with polyethylenimine was useful. Saturation studies demonstrated a high affinity component with a K_D value of approximately 0.7 nM, very similar to that reported previously with the tritiated material. Raising the concentration of β-endorphin further revealed additional binding sites with lower affinity (10-20 nM). Morphine and DADL-enkephalin at concentrations under 10 nM lowered [^{127}I]β_h-endorphin binding (0.2 nM) by about 25-30%. However, the inhibition of the two compounds was not additive. In the presence of one ligand, the other failed to lower binding. The remainder of the binding was more sensitive to β_h-endorphin than either of the other two ligands. DPDPE at concentrations up to 20 nM, over 5-fold higher than its K_D for delta receptors, failed to lower [^{127}I]β_h-endorphin binding at all, implying that at this concentration of radioligand no delta receptors were labeled. In summary, the results are similar to those with [^{3}H]β-endorphin. At low concentrations, the iodinated ligand appears to label two sites, one of which is probably mu_1 and the other probably epsilon. There is no evidence of delta labeling and mu_2 labeling also seems unlikely. Having characterized the binding of the iodinated ligand, we used this low concentration of [^{127}I]β_h-endorphin in crosslinking studies and ran SDS-PAGE gels. Our results are similar to those of other groups, with a major band at about 60-65,000 daltons and several additional bands corresponding to lower molecular weight species. However, our binding studies raise major questions regarding the published identification of these bands with specific classes of opioid receptors.

471.13 HUMAN ANTI-OPIATE RECEPTOR ANTIBODY BINDS TO RAT BRAIN PROTEINS. B.F. Roy*, W.D. Bowen, J.S. Frazier*, M.M. Dauphin*, S.B. Hellewell*, D.L. Murphy, and J.M. Morihisa (SPON: J.M. Morihisa). VA Medical Center, Psychiatry Service (116A), 50 Irving St. N.W., Washington, D.C. 20422

Sera from two patients with major depressive disorder and antibodies to β-endorphin (Roy, B.F., Proc. Natl. Acad. Sci., USA 83:8739, 1986) were shown to have anti-idiotypic antibodies which specifically inhibited binding between anti-β-endorphin IgG and β-endorphin. Autologous and homologous anti-idiotypic anti-anti-β-endorphin IgG antibodies were isolated by affinity chromatography binding to anti-β-endorphin IgG. Purified anti-idiotypic antibody did not bind β-endorphin. The effect of anti-idiotype on [^{125}-I]β-endorphin binding to rat brain membranes was determined. Antibody was dialyzed against distilled H_2O and incubated with a crude P_2 membrane fraction from rat brain in a buffer consisting of 50 mM potassium phosphate, pH 7.4 and protease inhibitors for 60 min at 25°C. Then 1 nM [^{125}-I]β-endorphin was added and incubation was carried out for an additional 60 min at 25°C. Incubations were terminated by filtration through glass fiber filters. Nonspecific binding was determined using 10 μM levallorphan. Autologous anti-idiotypic antibody reduced binding of [^{125}-I]β-endorphin to the P_2 membrane fraction of rat brain 89%, while homologous anti-idiotypic antibody reduced binding 70%. Normal IgG which was similarly treated had negligible competitive effects at equivalent concentrations. The idiotype specifically inhibited the ability of anti-idiotype to block [^{125}-I]β-endorphin binding to rat brain opiate receptor sites. These results indicate that the anti-idiotypic antibody binds to opiate receptors.

The ability of anti-idiotypic antibody to recognize membrane proteins was assessed by Western immunoblot analysis. Solubilized rat brain membrane proteins were resolved by two-dimensional polyacrylamide gel electrophoresis and blotted onto nitrocellulose. The blots were probed with autologous anti-idiotypic antibody followed by incubation with peroxidase-coupled goat anti-human IgG. A neutral protein of 60 kDa was detected. The molecular weight of this protein corresponds to that of the major rat brain protein labelled by iodinated β-endorphin in cross-linking studies (Howard, A.D., J. Biol. Chem., 260:10833, 1985). Additional bands of 116 and 110 kDa were detected in one-dimensional gels when [^{125}-I]Protein A and autoradiography were used as the method of visualization.

The anti-idiotype is potentially directed against receptors on T and B cells promoting the production of anti-β-endorphin antibody and may mediate interactions between parallel regulatory networks within the immune system and the CNS. Idiotypic networks for brain peptides may contribute to psychiatric disorders.

471.14 A MONOCLONAL ANTI-IDIOTYPIC ANTIBODY DIRECTED AGAINST A MONOCLONAL ANTI-MORPHINE ANTIBODY. R. Juszczak*[1], C.J. Coscia[1], M. Szücs*[2], M.R. Hanley[3] and E.A. Barnard*[3]. [1]Dept. of Biochem., St. Louis Univ. Sch. of Med., St. Louis, USA, [2]Inst. of Biochem., Biol. Res. Ctr. Hung. Acad. Sci., Szeged, Hungary, [3]Molec., Neurobiol. Unit, MRC Centre, Cambridge, U.K.

Anti-idiotypic, anti-receptor antibodies raised against antibodies to receptor ligands, provide a means of receptor isolation and characterization which circumvents initial receptor purification. We have generated an anti-idiotypic anti-opioid receptor antibody (Ab2) from monoclonal anti-morphine antibodies (mAb1) using conventional hybridoma techniques. The mAb1 used as antigen displayed stereospecificity and a greater affinity for opiate alkaloid agonists than antagonists. None of the enkephalins tested however, interacted with mAb1. Hybridoma culture supernatants were screened with a newly developed solid phase radioimmunoassay (RIA), based on the displacement of radiolabelled morphine from mAb1 with Ab2. One of the Ab2s that gave a positive RIA also competed with opioid ligands for rat brain opioid receptors. SDS-PAGE revealed the Ab2 to be highly purified after successive affinity and Protein A- Sepharose chromatography. In dose-dependency displacement studies, with either opiate alkaloids or opioid peptides as radioligand, this preparation displayed picomolar affinity for rat brain opioid receptors. Less than 13 μg mAb2 completely inhibited specific opioid radioligand binding to both soluble and membrane-bound opioid receptors. These results indicate that we have generated an anti-idiotypic antibody that specifically interacts with rat forebrain opioid receptors. Supported in part by a Fogarty Senior International Postdoctoral Fellowship (to CJC), NIH grant MH 40005 and NSF grant 85-08933.

471.15 EFFECTS OF FAB FRAGMENTS FROM A MONOCLONAL ANTIBODY DIRECTED AGAINST THE OPIOID RECEPTOR UPON THE BINDING OF σ RECEPTOR LIGANDS TO RAT NEURAL MEMBRANES. C.B. TYLER AND J.M. BIDLACK. Center for Brain Research, Univ. of Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

A monoclonal antibody, OR-689.2.4, directed against the opioid receptor inhibited μ and δ opioid binding to neural membranes but not κ binding (J. Biol. Chem., 261: 15844-15849, 1986). Previously, we reported that Fab fragments from this monoclonal antibody also inhibited the binding of the σ receptor ligand (+)[^{3}H]SKF 10,047 (Soc. Neurosci. Abstr., 12:407, 1986). These data suggested that μ, δ and σ receptors share a common structural component. Further studies have been conducted to more closely examine the two binding sites of (+)SKF 10,047, the haloperidol-sensitive and the PCP-sensitive sites. Binding at the haloperidol-sensitive was examined by using 1 nM [^{3}H]1,3-diortho-tolyl-guanidine (DTG) and binding at the PCP-sensitive site was assessed with 3 nM [^{3}H]N-1-[2-thienyl]cyclohexyl)-3,4-piperidine (TCP).

Rat neural membrane protein was incubated with OR-689.2.4 Fab fragments in a final volume of 1 ml in 50 mM Tris-HCl, pH 7.5, for either 60 min at 25°C or for 24 hr at 4°C. After this preincubation, ^{3}H-labeled ligands were added and the samples were incubated for 60 min at 25°C. Samples were filtered through Whatman GF/B glass fiber filters and washed with 10 ml ice-cold 50 mM Tris HCl, pH 7.5. In [^{3}H]TCP assays, the filter was soaked in 0.05% polyethylenimine for at least 60 min prior to filtration.

The antibody was not capable of inhibiting the binding to neural membranes of ^{3}H-labeled ligands specific for the β-adrenergic, benzodiazepine, nicotinic or muscarinic cholinergic receptors. These studies demonstrate the high specificity that the antibody has for the μ and δ opioid receptors and the σ receptor. As was seen with (+)[^{3}H]SKF 10,047, OR-689.2.4 Fab fragments inhibited the binding of [^{3}H]DTG and [^{3}H]TCP in a titratable manner. The Fab fragments were more effective in blocking the binding of [^{3}H]DTG than [^{3}H]TCP. With 1 μM OR-689.2.4 Fab fragments, the maximal inhibition of the binding of 1 nM [^{3}H]DTG increased from 33 ± 1.4% to 49 ± 1.2% with the longer preincubation period. For [^{3}H]TCP, however, maximal inhibition remained similar, 24 ± 0.9% vs 29 ± 2.2%, for the two preincubation time periods. The Fab fragments noncompetitively inhibited the binding of [^{3}H]DTG, suggesting that the antibody is not directed against the binding site. These data suggest that while they are distinct sites, the haloperidol-sensitive and the PCP-sensitive sites may have a common structural component.
(Supported by USPHS grants DA03742 and DA07232.)

471.16 KELATORPHAN, A POTENT ENKEPHALINASE INHIBITOR,AND OPIOID PEPTIDES DIFFERENTIALLY MODULATE SELF-STIMULATION BEHAVIOR FOLLOWING VENTRICLE OR ACCUMBENS MICROINJECTIONS. PH.De Witte, C. Heidreder* and B. Roques. Université de Louvain, 1348 Belgium ; Université Descartes, Paris.

The mesocortico-limbic dopaminergic system, originating from the ventral tegmental area (VTA) and projecting to a wide range of limbic structures including the frontal cortex, olfactory tubercles, lateral septum and nucleus accumbens is thought to have important functions in regulating central reward, exploratory, locomotion behavior and is even thought to be directly implicated in neuro-psychiatric disorders such as schizophrenia. Nucleus accumbens and VTA have been shown to display high concentrations of enkephalin and specific opiate receptors (delta and mu) have been identified in close association with both these structures. Kelatorphan, a potent inhibitor of enkephalin-degrading enzymes should help to elucidate the fonctionel role of endogenous enkephalins in the regulation of the dopaminergic neurons since opiates interact with enkephalin receptors which likely play an inhibitory action on dopamine transmission. We thus further investigate the behavioral effects and the common properties of dopamine agents such as amphetamine, haloperidol, apomorphine and opioid agents such as morphine, DLET, DAGO and kelatorphan on the intracranial self-stimulation behavior (ICSS) obtained through chronic electrodes implanted into the medial forebrain bundle (MFB) after lateral ventricle (ICV) or nucleus accumbens injections. The results indicate that kelatorphan (70 nM) and d-amphetamine (1 nM), directly ICV, induced an increase in ICSS lasting for 45 min. This facilitatory effect of MFB stimulation after kelatorphan injection was completely antagonized by 70 nM naloxone when co-administered. The ICV injection of morphine (2.5 nM) and mu opioid agonist DAGO (300 pM) led to a drastic ICSS decrease whereas 300 pM DLET (a delta agonist) significantly enhanced the ICSS rate which can be counteracted by 1.500 pM ICI 174864 (a delta antagonist) DAGO elicited a similar behavioral profile when administered into the nucleus accombens, i.e. a decrease in ICSS. DLET (1.000 pM), on the contrary, led to a slight non significative ICSS increase. Kelatorphan as well as d-amphetamine or apomorphine, induced a significant decrease in ICSS after accumbens injection. The microinjection of the neuroleptic haloperidol ICV or intra-accumbens strongly blocked the rate for ICSS additionally supporting the direct linkage existing between our electrode site excitement and dopamine release.

Our results clearly show that ICV or intra-accumbens injection of kelatorphan and amphetamine both induce the same pattern of increase (ICV) - decrease (accumbens) of rewarding brain stimulation showing thus that these two drugs share common rewarding properties. Furthermore the actions of kelatorphan resemble more closely to an action mediated by delta rather than mu opioid agonist.

71.17 THE EFFECTS OF pH ON THE POTENCY OF SELECTIVE INHIBITORS OF MEMBRANE-BOUND ENKEPHALINASE (endopeptidase 24.11) FROM RAT KIDNEY. J. Ansell*, M.D. Erion*, L.P. Wennogle, and A.Y. Jeng*. Research Department, Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, NJ 07901

Enkephalinase, a neutral metalloendopeptidase, hydrolyzes the Gly-Phe amide bond in Met-enkephalin (Tyr-Gly-Gly-Phe-Met) and in Leu-enkephaline (Tyr-Gly-Gly-Phe-Leu), which are potent biologically active peptides that can induce morphinomimetic effects. Hence, inhibitors of enkephalinase could have potential pharmacological importance. At present, the two most frequently used assays for enkephalinase are: (a) a radioactive assay, measured at pH 6.5, using [3H]-labeled Leu-enkephaline as the substrate (Altstein et al, Life Sci. 28, 185, 1981) and (b) a spectrophotometric assay, measured at pH 7.6, using Glutaryl-Ala-Ala-Phe-2-naphthylamide as the substrate (Orlowski and Wilk, Biochemistry 20, 4942, 1981). Depending on the assay system employed, literature values for the inhibition of enkephalinase by the two selective inhibitors, phosphoramidon and thiorphan, differ substantially. This prompted us to examine the effects of pH on the potency of phosphoramidon and thiorphan in the enkephalinase assay.

The crude membrane homogenate from rat kidney cortex was used as the enzyme source and the enzyme activity was determined spectrophotometrically. Tris buffers were adjusted to the desired pH at 37° at which all of the incubations were carried out. The membrane-bound enkephalinase was pre-incubated in 150 μL, containing 5 μg aminopeptidase M and 2.5 μmol Tris/HCl at the desired pH, for 10 min in the presence or absence of inhibitors. Glutaryl-Ala-Ala-Phe-2-naphthylamide (100 μL) was then added and incubated further for 10 min. The reaction was developed with Fast Garnet and the absorbance measured at 530 nm. Experiments performed at pH 7.4 in the absence of inhibitor gave a Km value of 710 ± 50 μM (mean ± SEM, n = 7). Subsequent competition experiments using phosphoramidon or thiorphan were done at a substrate concentration of 500 μM. The membrane-bound enkephalinase showed a broad pH optimum. The enzyme activities measured between pH 6.5 and pH 8.5 did not differ more than 25% from the maximal activity obtained at pH 7.4. However, the IC50 values for inhibitors varied significantly within the same pH range. For thiorphan, the IC50 values at pH 6.5, 7.4, and 8.5 were 4.1 ± 0.3 (4), 7.1 ± 0.8 (6), and 39 ± 9 (2) nM (mean ± SEM, (n)), respectively. More drastic variations were observed with phosphoramidon. At pH 6.5, 7.4, and 8.5, the IC50 values were 8.3 ± 0.9 (3), 80 ± 8 (4), and 1220 ± 20 (2) nM, respectively. Thus, care must be taken to define the incubation conditions in evaluating the potency of these inhibitors.

471.18 **COMPARISON OF SOLUBLE AND MEMBRANE-BOUND FORMS OF ENKEPHALIN-DEGRADING AMINOPEPTIDASE FROM RAT.** **S.H.Dyer* and L.B.Hersh. Department of Biochemistry, UTHSCD, Dallas, TX 75235-9038.**

Enkephalin degradation in brain has been shown to occur by both soluble and membrane bound puromycin sensitive aminopeptidases. These enzymes have previously been purified, although not in parallel, and appear similar in their catalytic properties. The relationship, if any, between the soluble and membrane bound enkephalin degrading aminopeptidases has not yet been established nor has the tissue specificity of this enzyme been examined.

We have recently purified both of these aminopeptidases from rat brain and have generated antiserum to the soluble aminopeptidase. Both enzymes were found to have the same molecular weight as determined by SDS-PAGE and size exclusion chromatography. The anti soluble aminopeptidase antiserum reacted with both enzyme forms on immunoblots and inhibited both enzyme forms with identical inhibition curves.

Studies on the distribution of soluble aminopeptidase activity in seven different rat tissues, using Ala-β-naphthylamide as substrate, showed that the puromycin sensitive aminopeptidase is the predominant activity in brain, heart and skeletal muscle. In spleen, liver and lung this activity accounted for approximately 50% of the total aminopeptidase activity, however kidney was devoid of this aminopeptidase.

These results establish a direct relationship between the soluble and membrane bound forms of the puromycin sensitive aminopeptidase and demonstrate that this enzyme, like other "enkephalinases" is not brain specific.

472.1 DISTRIBUTION OF INSULIN-LIKE GROWTH FACTOR II mRNA IN THE CENTRAL NERVOUS SYSTEM. K.M. Rosen* and L. Villa-Komaroff.
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Insulin-like growth factor II (IGF II) is a member of the family of peptides related to insulin by structure and function. Several lines of evidence suggest that IGF II plays a role in central nervous system development and maintenance. IGF II (and insulin) promote neurite outgrowth and enhance neuronal survival in culture in peripheral ganglion cultures from chick embryos (Bothwell, J Neuro Res 8:225,1982; Recio-Pinto et al J. Neurosci 6:1211, 1986). These peptides also affect neurite outgrowth and binding of nerve growth factor in the human neuroblastoma cell line SH-SY5Y (Recio-Pinto et al, PNAS 81:2562, 1984). Immunoreactive IGF II has been found in cerebral spinal fluid (Haselbacher and Humbel, Endocrinology 110:1822, 1982) and in several regions of the adult human brain. In adult human brain, the anterior pituitary has the largest amount of immunoreactive 7.5 kDa IGF II, with 6 fold more than any other region and 50 to 100 fold more than pons or cerebellum (Haselbacher et al, PNAS 82:2153, 1985). We have reported the isolation of a cDNA clone encoding IGF II from a human thalamus cDNA library (Irminger et al, PNAS, in press). The isolation of this clone demonstrates that authentic IGF II is synthesized by cells within the CNS. To determine the sites of synthesis of this peptide, we dissected brains from 60 day old animals into cerebrum, cerebellum, midbrain, pons plus medulla, thalamus and hypothalamus and prepared RNA from each region. A 90bp fragment of the human clone that has 93% homology to the rodent sequence was subcloned into the phage vector M13 to generate single-stranded probe of high specific activity. We annealled RNA from each region of the adult brain to the labeled probe, digested with S1 nuclease and ran the protected products on a high resolution polyacrylamide gel. Our results indicate that pituitary RNA contains 1% or less IGF II mRNA than does hypothalamic RNA, which is the richest source of IGF II in the adult brain. RNAs from thalamus and midbrain each contain about half the amount of IGF II RNA found in hypothalamus. RNAs from cerebrum and cerebellum contain about one tenth the amount of IGF II RNA found in hypothalamus. These results suggest that the IGF II found in the pituitary is not synthesized there. We are now trying to localize the main sites of IGF II synthesis within the hypothalamus.

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472.2 MOLECULAR CLONING, STRUCTURE AND DEVELOPMENTAL REGULATION OF AN ONCOFETAL POLYPEPTIDE OF MAMMALIAN NEURONS. A. K. Hall* and J. I. Morgan* (SPON: R. Chizzonite). Dept. of Neurosciences, Roche Institute of Molecular Biology, Nutley, NJ 07110.

Fetal and neonatal mammalian brain contain a unique polypeptide that has been purified by HPLC and sequenced by gas phase Edman degradation. The rat and human cDNAs encoding the molecule have been isolated and sequenced. Cloning of the human cDNA was facilitated by the discovery that most neuroblastomas also synthesize large amounts of the polypeptide. Using a 3' non-coding (EcoRI/AvrII) fragment of the rat cDNA it can be shown by *in situ* hybridization that the mRNA for the polypeptide is located specifically in neurons and not glial cells.

Developmental regulation of the oncofetal mRNA was evident in that it was most abundant in fetal and neonatal brain tissue. Levels of the mRNA in the brain declined to a nadir 36 days after parturesis. Elucidation of the tissue-specific expression of the gene was facilitated by analysis of various mouse tissues. The single 600 nucleotide mRNA species was found to be expressed albeit at very low levels when compared to fetal brain, in thymus, basal brain and spleen, trace amounts were also detected in lung, cerebral cortex, brain stem, cerebellum and liver. No specific mRNA was found in heart or renal tissue.

In related studies, we noticed that the initially high concentrations of the oncofetal peptide present in rapidly growing rat neuroblastoma cells, were markedly reduced when the cells were differentiated following treatment with dibutyryl cyclic AMP (dbcAMP). Use of the rat cDNA probe indicated that rat neuroblastoma cells also contained a single 600 nucleotide mRNA species thus further emphasizing the oncofetal neural nature of the polypeptide.

Together, these findings lead us to speculate that this oncofetal polypeptide may play a role in the mechanisms which modulate growth and differentiation of mammalian neuronal cells. Present studies are directed towards identifying the promoter/enhancer elements of the gene encoding the polypeptide. These genomic sequences will be compared with the regulatory element of N-myc which shows a similar expression in fetal brain and neuroblastomas.

472.3 DISTRIBUTION OF PROTO-ONCOGENE EXPRESSION IN RAT BRAIN. A.M. Foote*, N.Rost*, C. Chauvin*, M.F. Nissou* and A.L. Benabid.
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Proto-oncogenes, the cellular homologues of the transforming oncogenes are believed to code for proteins involved in the control of cellular growth and differentiation (Bishop,J.M., Am.Rev.Biochem.,52:301,1983). Certain proto-oncogenes, c-fos, have even been implicated in the processes leading to the development of long-term memory (Goelet,P. et al., Nature, 322:419,1986). Others, erbA, erbB, sis, are known to be involved in signal transduction, a process of evident importance in the brain. Thus, it is highly likely that some proto-oncogenes will exhibit a distribution of expression in the developing and possibly in adult brain. For example: Ruppert et al.(EMBO J.,5:1897, 1986) have recently demonstrated elevated c-myc expression in granule cells of the external matrix layer and in Purkinje cell somata of the developing murine cerebellum.

Our aim is to further understanding of the origin of human brain tumors and to identify sites which may be more susceptible than others to malignant transformation due to inherently increased levels of expression of certain proto-oncogenes. We are investigating the distribution of expression of a selection of proto-oncogenes in rat brain, adult and during post-natal development, by in situ hybridization.

In situ hybridization was performed essentially by the method of Bloch et al. (J.Neurosciences Res.,1987), using oncogene specific c-DNA sequences labelled with ^{35}S by nick translation to 2×10^8 cpm/ug. In order to verify the specificity of the signal observed positive and negative controls were performed on 15µm formaldehyde fixed brain sections serial with those hybridized with the oncogene probe. c-DNAs for rat pre-proenkephalin A and rat transferrin were used as positive controls, both exhibiting a distinct distribution of expression and translation in brain, as demonstrated by in situ hybridization and immunohistochemistry. Negative controls, which eliminated the signal, included pre-treatment with RNAse and pre-saturation of the mRNA with unlabelled probe. The relative levels of mRNA in the various brain regions were determined by digital analysis of the autoradiographs against Northern hybridization controls using the same probe batch.

mRNAs hybridizing with c-myc and N-myc, proposed to code for nuclear DNA binding proteins, appear only at very low levels in the adult rat brain and no distinct pattern of gene expression was observed. Similarily, Ha-ras, coding probably for a G protein involved in membrane signal transduction, although expressed in adult rat brain, is not more highly expressed in any particular loci than elsewhere. In contrast, c-sis, shown to be a percusor of the ß chain of PDGF (Johnsson et al., EMBO J., 3: 921, 1984). appears to be highly expressed in the cerebellum and in the anterior commissure of the adult rat as shown by hybridization of a 0.83 Kb Pst I-XBa I probe from cloned SSV. Likewise, preliminary results indicate that the proto-oncogenes coding for cell surface receptors, erbA (thyroid hormone receptor) and erb B (EGF receptor) are also more highly expressed in the cerebellun than elsewhere in the adult rat brain. This work is supported by an EMBO long term fellowhip to AMF; INSERM, ESPOIR, LCC and FLCC.

472.4 THE DIFFERENTIAL EXPRESSION OF α-TUBULIN GENES DURING NEURONAL DEVELOPMENT IS REITERATED DURING NEURONAL REGENERATION. F.D. Miller*, W. Tetzlaff, C.C.G. Naus, M. Bisby, F.E. Bloom, R.J. Milner. Res. Inst. of Scripps Clinic, La Jolla, CA, and Univ. of Calgary, Calgary, Canada.

We are interested in the possible recapitulation of developmentally-specific patterns of gene expression during neuronal regeneration in the mature nervous system. Two different mRNAs for α-tubulin that encode almost identical proteins, termed Tα1 and T26, are known to be expressed in the embryonic rat nervous system. We have compared the patterns of expression of these two α-tubulin mRNAs during neural development and during regeneration of the facial (VII) nerve using Northern blotting, ribonuclease protection, and in situ hybridization techniques. These experiments indicate that Tα1 mRNA is at least ten-fold more abundant in the embryonic nervous system than any other α-tubulin mRNA. Tα1 mRNA is highly enriched in the embryonic nervous system compared to non-neural tissues but is much less abundant in the adult brain; T26 mRNA is expressed in many embryonic tissues with little change in abundance in the nervous system during development. Within the developing nervous system, Tα1 mRNA is enriched in regions such as the cortical plate, which contain neurons undergoing neurite extension, while T26 is relatively homogeneous in its distribution, with some enrichment in proliferative zones. Expression of Tα1, but not T26, mRNA is increased in PC12 pheochomocytoma cells induced to differentiate and extend neuritic processes by nerve growth factor. Taken together, the data indicate that the Tα1 α-tubulin mRNA is expressed at high levels during the developmental extension of neuronal processes.

To determine whether this specific developmental expression of Tα1 α-tubulin mRNA could be reinduced in the mature nervous system, we have examined the expression of Tα1 vs T26 mRNAs in unilateral, extracranial crushes of the facial nerve. Northern blot and ribonuclease protection experiments demonstrated that Tα1 mRNA is induced during regeneration of facial motor neurons, whereas T26 mRNA levels remain constant. In situ hybridization analysis confirmed this differential induction, and further demonstrated that the increase in Tα1 mRNA levels is specific to motor neurons of the facial nucleus, and not to proliferating glial cells. These data therefore demonstrate that Tα1 α-tubulin mRNA expression is tied to the extension of neuronal processes in the mature nervous system as well as in the developing nervous system, and that the developmental pattern of α-tubulin gene expression is reiterated in regenerating motor neurons. Supported in part by grants from the NIH (NS 22347), NIAAA (AA06420), AHFMR, Canadian MRC, and McNeil Pharmaceuticals.

472.5 NEUROFILAMENT MESSANGER RNA LEVELS DECREASE FOLLOWING SCIATIC NERVE TRANSECTION. M.E. Goldstein, S. Weiss, R. Lazzarini, P. Shneidman, J. Lees and W.W. Schlaepfer. Lab. of Neuropathology, Univ. of Pennsylvania Med. Sch., Philadelphia, PA 19104.

Total RNA was isolated from the left (experimental) and right (control) dorsal root ganglia of Sprague-Dawley rats 3, 7, 14, 21, and 28 days after transection of the left sciatic nerve. Levels of RNA coding for the neurofilament triplet proteins (NF-H, NF-M, and NF-L) were analyzed on Northern and dot blots using ^{32}P-labeled cDNA probes specific for each of the proteins. Autoradiograms reveal a decrease in the RNA level for all three subunits as early as 3 days following transection and continuing for at least 28 days. The results indicate that neurofilament protein synthesis following nerve injury is regulated at the level of transcription.

472.6 MOLECULAR CLONING OF GENES INVOLVED IN PERIPHERAL NERVE REGENERATION. H.Schaal[1*], P.Spreyer[1*], A.Unterbeck[2*], K.Olek[3*], B.Müller-Hill[2*] and H.W.Müller[1] (SPON: W.Seifert). [1]Molecular Neurobiology Laboratory, Dept. of Neurology, University of Düsseldorf,F.R.G., [2]Inst. of Genetics, University of Köln, F.R.G., [3]Inst. of Radiation Biology, University of Bonn, F.R.G.

Following injury of peripheral nerves in mammals the expression of a small group of proteins is sequentially induced or inhibited in the distal nerve stump during Wallerian degeneration and axonal regrowth suggesting the induction/repression of specific genes in nerve repair.

In an attempt to identify genes regulated in the regeneration program of rat peripheral nerve we have prepared poly(A)$^+$-RNA from mature normal and regenerating (1 week post crush) rat sciatic nerve, respectively. Following the procedure of Okayama & Berg a cDNA library was constructed from poly(A)$^+$-RNA of regenerating nerves and 2ooo clones were prepared as permanent glycerol stocks in microtiter plates. These colonies were screened by differntial filter hybridization using ^{32}P-labeled single strand cDNA from regenerating and normal rat sciatic nerve poly(A)$^+$-RNA as probes.

Further investigation by Northern blotting and sequence analysis of differntially expressed cDNA clones with strong hybridization signals revealed that e.g. P_o (major glycoprotein of peripheral nerve myelin) is repressed at one week post crush while actin, vimentin, and EF-1a (elongation factor) are highly induced in cells of the nerve sheath. The identified cDNA clones were used to estimate the steady state levels of mRNAs homologous to cDNA sequences which had yielded less intense hybridization signals and could not be identified by VAX/VMS computer analysis. The pattern of differential gene expression observed after nerve injury will be presented.

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472.7 REGULATION OF RNAs ENCODING THE 28 kD CALCIUM BINDING PROTEIN IN RAT KIDNEY AND CEREBELLUM. T.L. Wood*[1], A.J. Tobin[1,2,3], and S. Christakos*[4] (SPON: A. Beyer-Mears). [1]Department of Biology, [2]Molecular Biology Institute, and [3]Brain Research Institute, University of California, Los Angeles, CA 90024; and [4]Department of Biochemsitry, New Jersey Medical School, Newark, NJ 07103.

We have studied the regulation of the RNAs encoding the 28 kD calcium binding protein (CaBP or Calbindin D_{28k}) in rat kidney and cerebellum, using our mouse cDNA from a lambda gt-11 library (Wood, et al., in preparation). In mammals CaBP is present at high concentrations in specific cells of both kidney and brain. It is particularly abundant in the cerebellum, where it represents about 1.5% of the total soluble protein and is localized entirely in the Purkinje neurons.

CaBP is thought to play a role in calcium homeostasis in the kidney, where it is induced by vitamin D and by low dietary phosphorus. The role of CaBP in the brain is unknown.

We examined the levels of CaBP RNAs in kidneys and cerebella of rats raised on diets known to change the concentration of CaBP in the kidney. These diets included: (1) control, vitamin D replete; (2) minus Ca^{+2}, vitamin D replete; (3) minus phosphorus, vitamin D replete; (4) high vitamin D; (5) minus Ca^{+2}, high vitamin D; (6) minus phosphorus, high vitamin D; and (7) vitamin D-deficient.

We analyzed kidney and cerebellar CaBP RNAs on Northern blots. Low dietary calcium decreases CaBP RNAs in both kidney and cerebellum. Low dietary phosphorus increases CaBP RNAs in the kidney but not in the cerebellum. Rats raised with high dietary vitamin D have increased levels of CaBP RNAs in the kidney, but decreased levels in the cerebellum. In rats raised on vitamin D deficient diets, CaBP RNAs are decreased in the kidney but unchanged in the cerebellum.

The regulation of CaBP RNAs differs in rat kidney and cerebellum. Although the kidney and cerebellum both have reduced CaBP RNAs in response to low dietary calcium, their response to dietary phosphorus and vitamin D are vastly different. Different repsonses to environmental cues may reflect different functions of CaBP in brain and kidney.

This work was supported by grants from NINCDS to AJT (#NS 20356 and 22256) and to SC (#NS 20270) and from NSF to SC (#PCM 84-02840). TLW was supported in part by USPHS Training Grant in Integrative Biology (#GM 07191).

472.8 MOLECULAR GENETIC ANALYSIS OF PEP-19 EXPRESSION IN DEVELOPING MAMMALIAN BRAIN. L. Sangameswaran* and J. I. Morgan* (SPON: S. Korn). Dept. of Neurosciences, Roche Institute of Molecular Biology, Nutley, NJ 07110.

PEP-19 is a 7.6 kDa polypeptide that was discovered in our laboratory as a developmentally regulated neuropeptide from rat brain. Although it has a unique amino acid sequence, it shares a homology with the calcium binding domains of several calcium binding proteins such as S-100, parvalbumin, calmodulin, calbindin and troponin. Thus, PEP-19 may be considered as a small polypeptide with a single calcium binding domain. By HPLC analysis, it was shown that PEP-19 is enriched in the cerebellum and olfactory bulb with smaller amounts present in other regions of the brain. It is localized in Purkinje cells and stellate neurons of the cerebellum as observed by immunocytochemistry with a site-directed polyclonal antibody.

We have recently cloned PEP-19 from a λgt11 library constructed with poly (A^+) mRNA from adult rat brain. 18 positive clones were picked after screening 150,000 plaques with the antibody. One of them, 20b.2, with the longest insert (504bp) was subcloned into pBR322 and sequenced by the method of Maxam and Gilbert. It contains the entire coding region. The amino acid sequence deduced from the cDNA sequence fitted perfectly with that from the polypeptide. The coding region which is the only open reading frame is followed by 276 neucleotides 3' untranslated region.

This clone was used as a probe to determine if PEP-19 mRNA, like the polypeptide is under developmental regulation in rat brain. The mRNA is detectable as early as embryonic day 17 and increases with age reaching a maximum at 18 days postpartum. We also analyzed mRNA from different regions of the brain as well as several non-neuronal tissues. There is a single species of message in cortex, basal brain, spinal cord, brain stem, olfactory bulb and retina. However, it is undetectable in a variety of nonneuronal tissues tested confirming that this polypeptide is exclusively neural in distribution.

The cDNA probe was also used to investigate the mRNA level in cerebellar mutant-mice in an attempt to employ it as a marker to study cerebellar development. The mutants used in our investigation included nervous, reeler, staggerer, pcd, weaver and lurcher. These data will be discussed in relation to cerebellar development.

472.9 CELLULAR DISTRIBUTION OF RNAs ENCODING GAD AND THE 28 kD CALCIUM BINDING PROTEIN IN THE BRAINS OF MICE, RATS, AND GERBILS. G.D. Frantz*[1], S. Feldblum*[1], C.W. Wuenschell[1], J.H. Menkes*[2,3], and A.J. Tobin[1,4,5] (SPON: M. Wexler). Departments of [1]Biology, [2]Pediatrics, and [3]Neurology, [4]Molecular Biology Institute, and [5]Brain Research Institute, University of California, Los Angeles, CA 90024.

We are using in situ hybridization to ^{35}S-labeled antisense RNAs to determine the cellular distribution of the mRNAs encoding glutamic acid decarboxylase (GAD) and the 28 kD calcium binding protein (CaBP or Calbindin D_{28k}) in the brains of mice, rats, and gerbils. These studies address two hypotheses: (1) that altered cellular environments lead to changes in the expression of these two genes; and (2) that increased seizure susceptibility in seizure sensitive gerbils results from the altered expression of these two genes in the hippocampus and substantia nigra.

In normal mice, GAD mRNA is found in especially high concentrations in specific cell types in the following brain regions: cerebellum, hippocampus, reticular nucleus of the thalamus, cortex, inferior and superior colliculi, substantia nigra pars reticulata, globus pallidus, septum, diagonal band, olfactory bulb, and zona incerta. In normal mice and rats, CaBP mRNA is found in especially high concentrations in cells in the following regions: cerebellum, hippocampus, dentate gyrus, inferior olive, reticular formation, olfactory bulb, entorhinal cortex, caudate nucleus, amygdala, and vestibular nuclei.

GAD and CaBP mRNAs are expressed in distinct but overlapping neuron types. In the cerebellum, for example, Purkinje neurons contain both GAD and CaBP mRNAs. Basket, stellate and Golgi II neurons, however, contain GAD mRNA but not CaBP mRNA. In the hippocampus, GAD mRNA is present in a small population of interneurons in all layers, while CaBP mRNA is present at high concentration in the pyramidal cells of CA1 and CA2. The granule cells of the dentate gyrus contain high levels of CaBP mRNA, but no detectable GAD mRNA.

We are now studying the developmental regulation of these mRNAs in normal rodents and in ataxic and seizure susceptible mutants.

This work was supported by a grant to AJT from NINCDS (#NS 22256) and a program project grant to Dr. A.V. Delgado-Escueta (#NS 21908). GDF is supported by a USPHS Training Grant in Cell and Molecular Biology (#GM 07185).

472.10 STRUCTURE AND REGULATION OF MuBr8, A GENE EXPRESSED IN SPECIFIC NEURONAL CELL GROUPS. G.A. Oyler *2, G.A. Higgins *1, and M.C. Wilson *1 (Spon: R.A. Hawkins) [1]Research Institute of Scripps Clinic, La Jolla, CA 92037 and [2]Hershey Medical Center of Penn State, Hershey, PA 17033.

Investigations into the regulation of neuronal specific genes has been undertaken with the goal of elucidating the mechanisms that may account for the higher complexity of both cell type and accompanying gene expression in the brain. A set of cDNA probes to mRNA's which are highly expressed in the neurons of the murine hippocampal formation (HF) have been identified. The HF has been chosen as a model of neuronal specific gene expression because it has a well defined cytoarchitectonic organization, synaptic circuitry and electrophysiological responses. Thus, the HF is well suited for the examination of patterns of specific gene expression in the normal animal, during development and in disease states. One mRNA, MuBr8, has previously been shown by in situ hybridization to be abundantly expressed in the pyramidal cells of the CA3 field of the HF relative to other CA fields and dentate gyrus. This 2.2kb mRNA is also selectively expressed in other limbic system structures such as the anterior thalmus and mammalliary bodies as well as cerebral cortex, deep cerebellar nuclei and pons. MuBr8 is expressed in purified neuroblastoma C1300 cells induced to differentiate by contact growth inhibitor, serum deprivation and DMSO treatment. To determine the protein encoded by this mRNA a series of overlapping clones and a full length cDNA for MuBr8 have been isolated and sequenced. An initial search of regions containing translational open reading frames has not revealed identity with any known proteins suggesting that MuBr8 encodes a novel polypeptide, specifically and abundantly expressed by a subset of neurons in rodent brain.

To focus on regulatory elements governing expression of MuBr8 mRNA, genomicclones spanning at least 18kb of the gene have been isolated and mapped for restriction sites. Synthetic oligonucleotides from the cDNA have been used to identify the 5' transcriptional start site and thus indicate adjacent upstream regions in the cloned genomic DNA containing putative regulatory sequences. Transient transfection assays using differentiated C1300 cells as an expression system will be combined with deletional mutagenesis of plasmids containing the 5' upstream sequences to characterize important regulatory elements of MuBr8. Supported by American Heart Association (G.A.O.), Hereditary Disease Foundation Fellowship (G.A.H.) and NIH grant NS23038 (M.C.W.).

472.11 CHARACTERIZATION OF TWO RARE POLYA$^-$ mRNAS OF RAT BRAIN. B.P. Fung, M.H. Brilliant and D.M. Chikaraishi, Neuroscience Program, Tufts University School of Medicine, Boston, MA 02111 and Jackson Laboratories, Bar Harbor, ME 04609

The population of high-complexity RNAs expressed in the adult rodent brain has been shown to fall in nearly equal proportions into two classes: polyadenylated (polyA$^+$) and non-polyadenylated (polyA$^-$). Since the brain appears to be unique among organs in having high numbers of polyA$^-$ mRNAs, it is attractive to speculate that this peculiar population of messages has an important role in establishing the complex character and function of the brain. We have used molecular cloning techniques to isolate genomic sequences that are expressed only in brain, and two of these genomic clones, rg13 and rg100, have been investigated in some detail. Both are single-copy DNA genes and represent rare polyA$^-$ transcripts. In addition, both are developmentally regulated. Transcripts of rg100 are present at very low levels at embryonic day 17 (E17), and increase with developmental age. The level of rg100 expression at postnatal day 10 is equivalent to that in adult brain. On the other hand, the expression of rg13 appears to be slightly greater at E17 than at embryonic day 20 or at birth; again, by postnatal day 10 the adult level is present. The genomic sequences rg13 and rg100 thus appear to be under similar, but slightly different regulatory programs. In general, these data agree with previous observations, from this laboratory as well as others (Chaudhari and Hahn (1983) Science 220:924), that most of the polyA$^-$ transcripts arise postnatally whereas most polyA$^+$ mRNAs are already present at birth. These clones may, therefore, be members of a subclass of genes which are important in the late stages of brain development and/or in some specific brain function(s) which becomes fully established only after birth.

Although $\geq$ 90% of the rg13 and rg100 transcripts in the adult brain fractionates as polyA$^-$ on oligo-dT cellulose columns, less than 10% reproducibly fractionates with the polyA$^+$ population, detected by transcription mapping assays. While the amount of rg100 and rg 13 polyA$^-$ mRNAs increases with developmental time, the amount in the polyA$^+$ fraction remains constant. It may be that some development-associated change occurs, such as in the stability of polyA$^-$ transcripts, that results in the accumulation of a postnatal pool of non-adenylated mRNAs. At present, we do not know if the polyA$^+$ and polyA$^-$ forms of the clone-related transcripts might arise from distinct transcription or processing events (e.g., alternative 3'-end choice), or for example from the breakdown of a very long precursor. The phenomenon nonetheless appears to be unique to the brain.

472.12 SPECIFIC mRNA IDENTIFIED DURING DEVELOPMENT IN MOUSE PURKINJE CELLS AND THEIR DENDRITES. F. Levinthal*, J. Oberdick*, S.M. Yang* & C. Levinthal. Dept. Biological Sciences, Columbia Univ., New York, NY 10027

We have used in situ hybridization to study gene control of cell morphology and function during early development. Our efforts were concentrated on gene expression in the mouse cerebellum where the bulk of the development takes place postnatally between day 8 and 18.

A cDNA library in λgt10 was constructed with total polyA RNA, extracted at day 13 after birth from wild type cerebellum. It was enriched for Purkinje mRNA's by screening duplicate colonies with wild type and Lurcher RNA preparations (see abstract at this meeting by J. Oberdick, et al. "L7 is a marker for the terminal differentiation of Purkinje cells.").

In situ hybridizations were performed on cerebellum sections from wild type mice at different ages after birth: day 1,8,10,14 and 21. The probe,in the experiments reported here, was a nick-translated, tritiated cDNA from a clone chosen for its decreased expression in the Lurcher compared to the wild type (L7). Silver grains generated by radioactive decay on a NTB-2 emulsion were counted, over different regions of the brain.

The hybridization is localized in Purkinje cells and their dendrites. There is no labelling found in newborn and the counts are maxima at day 8 and 10. Although still present at day 21, they are reduced in the cell bodies. When the silver grains were counted per Purkinje cell and per dendritic tree, the counts per cell are maximum at 8 days: 79 counts versus 68 at 10 days and 35 at 21 days. In the dendritic tree, the counts are roughly constant: 48 - 43 - 40 counts. All numbers represent average counts of 10 cells.

At age 8 or 10 days, a number of Purkinje cells can be found in the granular cell layer; they are the cells which have not yet reached their final destination and they have not started sprouting their dendrites. They show no label.

We have other clones which hybridize preferentially with Purkinje cells, although not as specifically as L7. Preliminary results indicate that they do not hybridize with the dendritic processes.

L7 represents a DNA sequence involved specifically in the development of Purkinje cells.

Experiments are in progress to identify at an E.M. level the localization of the mRNA in the dendrites and several other clones are also being studied.

472.13 EXPESSION OF MYELIN PROTEOLIPID PROTEINS (PLP AND DM-20) IN THE DEVELOPING HUMAN SPINAL CORD. K.E. Kronquist*, B.F. Crandall*, W.B. Macklin, and A.T. Campagnoni, Mental Retardation Research Center, U.C.L.A. School of Medicine, Los Angeles, CA 90024.

We have cloned and sequenced a 2956 bp cDNA that encodes human myelin proteolipid protein (PLP) and have used this clone to examine PLP mRNA expression in developing human spinal cord between 11 and 24 weeks after conception. Fetal tissues were obtained within minutes of elective abortions, frozen immediately in liquid nitrogen, and stored at -80°C. Poly(A)$^+$ RNA was isolated from pooled human spinal cord obtained at three periods of development: 11-14 weeks, 17-19 weeks, and 21-23 weeks. Northern blots containing equal amounts of RNA were examined with a fragment of the PLP cDNA that contained the entire PLP coding region and 450 bp of the 3'-untranslated region. Several size classes of PLP mRNA were detected, the major bands being at 3.2, 2.7 and 2.3 kb with minor bands at 1.95, 1.5 and 1.2 kb. The abundance of the 3.2 kb RNA increased with age. Densitometric analysis indicated that, relative to the 11-14 week group, the 3.2 kb band was enriched 2- fold in the 17-19 week age group and 20-fold in the 21-23 week age group, at which point it represented 98% of the total integrated area of the PLP-hybridizing bands. In the 11-14 and 17-19 week age groups, the 3.2 kb band represented 36% and 12%, respectively, because of the complex expression of the smaller mRNAs. The PLP-hybridizing band at 2.9 kb also appeared to increase with age but this component was relatively minor. The 2.3 kb band was most prominent in the 17-19 week age group where it represented approximately 70% of the total integrated area of PLP-hybridizing bands. Examination PLP mRNAs in total cellular RNA isolated from individual spinal cords confirmed the age-dependent increase of the 3.2 kb PLP mRNA and indicated that the prominent 2.3 kb band emerged at around 16 weeks. Some of the smaller bands may represent mRNAs encoding DM-20, a myelin protein closely related toPLP. Other studies suggest that DM-20 mRNAs may have a protein coding sequence identical to PLP except for an internal deletion. The smaller RNAs may also arise from the utilization of alternate polyadenylation signals; there are six such signals in the 3'-untranslated region of the human PLP cDNA. Immunoblot analysis of individual spinal cord homogenates indicated that polypeptides for PLP and DM-20 protein could be detected at 18-21 weeks of conceptional age whereas polypeptides for myelin basic protein were present 3 to 4 weeks earlier. Supported by PHS grants NS 23022, NS 23322, and RR5756.

472.14 CHARACTERIZATION OF GAP JUNCTION MESSENGER RNA IN CULTURED GLIA AND NEURONS. K. Spiegel, David C. Spray and J.A. Kessler. Depts. Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Gap junctions are expressed in both the developing rat nervous system and in certain populations of neurons and glia in the mature brain. The present study characterizes messenger RNAs (mRNAs) for the gap junction that are expressed by cultured glia and neurons. Formation of electrotonic synapses is influenced by the environment of the neuron. For example, treatment of cultured sympathetic neurons with insulin or cAMP analogs promoted formation of electrotonic synapses (Kessler et al, PNAS 81:6235, 1984). Coupling of neurons after these treatments was prevented by inhibitors of RNA synthesis, suggesting that gap junction gene transcription was required. The precise nature of the gap junction gene and its messenger RNAs in the nervous system is unknown. However, the recent isolation of a cDNA for the liver gap junction provided a tool for examining gap junction mRNA regulation in the nervous system. Accordingly, cultures of embryonic (E17) striatal astrocytes and cultures of neonatal sympathetic neurons were examined for gap junction mRNA using a single-stranded antisense RNA liver gap junction probe and Northern blot analysis. Hybridization of Northern blots from cultured glia revealed a high molecular weight (MW) band of approximately 3.7 kb, a prominent, lower MW band, and a third faint band of even lower MW (approximately 1.6 kb). Glia cultured under these conditions expressed both dye and electrotonic coupling. By contrast, cultured sympathetic neurons contained a similar high MW band and a prominent band at approximately 1.6 kb, but no intermediate size band. Under these culture conditons, neurons were not coupled. Further studies will determine whether there is a correlation between expression of the intermediate MW mRNA species and formation of functional gap junctions and will determine the degree of homology between liver and nervous system gap junction mRNAs.

472.15 ALPHA-1-ACID GLYCOPROTEIN GENE EXPRESSION IN NORMOTENSIVE AND HYPERTENSIVE RATS. M.E. Norvitch*, V. Gold* and L.R. Meyerson. American Cyanamid Co., Med. Res. Div., Ramapo College, Mahwah, NJ 07430.

The modulation of serotonin (5-HT) transport has important ramifications in both the periphery and the brain as related to vasomotor tone, gut motility and neural information transfer. The transporter that regulates 5-HT availability in the human platelet is allosterically coupled to the site labeled by [3H]imipramine (J.Neurochem 48:560,1987). A natural protein modulator was purified from human plasma which not only competes for this allosteric site but enhances 5-HT uptake activity as well (BBA 923:8, 1987). This macromolecule was identified as alpha-1-acid glycoprotein (AGP). The exact function of AGP to date remains obscure. We studied general aspects of AGP gene expression in the liver and brain of normotensive (Kyoto-Wistar) and spontaneously hypertensive (SHR) rats to determine whether AGP gene transcription or mRNA translation were altered as a consequence of genetic hypertension. AGP gene transcription rates, abundance of AGP mRNA within steady-state total cellular RNA populations and protein synthetic activities were determined. The results demonstrate clear differences in AGP gene expression between these two strains. The relative abundance of AGP mRNA within both hepatic and brain RNA populations is approximately 3-fold greater in the SHR compared to Kyoto-Wistar controls. The ability of the normotensive and hypertensive rats to stimulate AGP production following turpentine or dexamethasone administration was also examined. Preliminary data suggest that the level of hepatic AGP mRNA induction is less in the SHR than in the Kyoto-Wistar (4- versus 5- fold, respectively). The levels of AGP mRNA within a variety of neural and non-neural tissues were investigated. The abundance of AGP mRNA within steady-state total cellular RNA populations was greatest in the hippocampus, followed by corpus striatum, liver, hypothalamus, thyroid, pons, medulla, stomach, aorta, pineal gland, spinal cord, heart, cerebellum and cortex. The AGP mRNA level in hippocampus (70%) rivaled that observed in turpentine-induced liver (100%). Collectively, these data indicate that AGP gene expression is altered in both brain and liver in SHR's compared to normotensive controls. Studies on how this altered expression in the SHR affects 5-HT physiology are underway.

472.16 THE DIFFERENTIAL INDUCTION OF TWO hsp70 mRNAs IN SEVERAL REGIONS OF RAT BRAIN BY D-METHAMPHETAMINE. E.K. Miller*, M.E. Trulson, G.I. Mues*, T.Z. Munn*, M.L. Goggans*, M.R. Morrison, and J.D. Raese (SPON: J. Steinberg). Departments of Neurology, Biochemistry, and Psychiatry, University of Texas Health Science Center at Dallas, Dallas, Texas, 75235, Schizophrenia Research Center, Veterans Administration Medical Center, Dallas, Texas, 75216, and Department of Anatomy, College of Medicine, Texas A & M University, College Station, Texas, 77843.

The 70 kd heat shock protein gene family contains genes that are transcribed in unstressed cells, the heat shock cognate 70 genes (hsc70), and genes whose transcription is induced by stress, heat shock protein 70 (hsp70) genes. This family also includes primate-specific genes that are transcribed in unstressed cells but that are induced many-fold by heat (hsx70). In this study, hsc70 mRNAs were identified by hybridization to a rat hsc70 genomic clone that hybridizes only to hsc70 mRNAs. The hsp70 mRNAs were identified by hybridization to an oligodeoxynucleotide probe synthesized against a region of the human hsx70 mRNA (hsp70 probe), that hybridized to hsp70 but not to hsc70 mRNAs.

Northern hybridization of total RNA from adult rat brain showed that the hsc70 probe hybridized to a 2.5 kb mRNA present in the 4 brain regions analyzed (cerebral cortex, cerebellum, caudate nucleus and midbrain). There was no detectable hybridization to the hsp70 probe. In heat shocked adult rats, the levels of the hsc70 mRNA were not altered and the hsp70 probe hybridized to 2 mRNAs, 2.9 and 3.1 kb in size (hsp70 mRNAs). A single IP dose of D-methamphetamine (10 mg/kg body weight) increased the body temperature of adult rats by several degrees over a 2 hour interval. This treatment also induced the 2 hsp70 mRNAs. As in hyperthermia, the hsc70 mRNAs levels were not altered. The temporal response to D-methamphetamine was quantitated in the 4 brain regions by slot blot hybridization to the hsp70 probe. The results showed that the extent of the hsp70 mRNA induction varied between animals, especially at the early time points. Despite the variation seen between animals, the time course and the magnitude of response, for each brain region, were reproducibly different. 30 minutes after injection, hsp70 mRNA levels were 2-10 fold higher in the cerebellum than in the cerebral cortex. 60 and 120 minutes after injection, hsp70 mRNAs were still most abundant in cerebellum. Lowest levels were found in the cerebral cortex while the midbrain and caudate nucleus had intermediate values. Two possible modes of action, that are independent but not mutually exclusive can be postulated to explain these differential kinetics. D-methamphetamine could act directly and differentially on the cells in the 4 brain areas examined. Alternatively, the cells of these brain area could response differentially to hyperthermia.

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472.17 CARBAMAZEPINE STUDIES ON BRAIN PROTEIN SYNTHESIS. S. Tewari*, W.E. Bunney, D. Winn*, and S. Patel*. Dept. of Psychiatry and Human Behavior, California College of Medicine, University of California, Irvine, Irvine, CA 92717.

Carbamazepine (CBZ) is an anticonvulsant drug with a structure similar to antidepressants. Although effective in treating manic-depressives, to date the mechanism by which CBZ exerts its antidepressant effects remains unclear. Studies from our laboratory have demonstrated that CNS depressants like ethanol or barbiturates, given acutely or chronically, produce inhibitory effects on brain protein synthesis. On the other hand, lithium carbonate well recognized for its antimanic and antidepressant effects specifically stimulated the protein and RNA synthetic activities in the brain both acutely and chronically, while inhibiting in the kidney and liver at the same time. To date, no data on CBZ are available on the protein biosynthetic processes in the cerebral tissue which is a sensitive target for drug action; regulation of protein and RNA biosynthetic processes plays an integral role in the cerebral functional processes and is consistant with its heterogeneity. Presently, in order to explain the possible mechanism of the antidepressant effects of CBZ, we have determined whether protein synthetic processes in the brain are influenced by acute or chronic exposure to CBZ. Two month old male Sprague-Dawley rats were intragastrically intubated with either 50mg/Kg CBZ suspended in sesame oil or with sesame oil (vehicle) only. The route of intragastric administration was chosen to closely approximate the oral intake under the clinical conditions. Experiments were carried out for acute studies at 30 min and 90 min after CBZ administration, and for chronic studies after two weeks of daily exposure to a single dose of 50mg/Kg CBZ. Protein synthesis was measured by translating in vitro free and bound brain polysomes in the presence of ^{35}(S) methionine under conditions where: (1) the incubating reticulocyte lysate medium was depleted of messenger RNA by micrococcal nuclease treatment and (2) also in a endogenous self supporting soluble factors supplemented medium. Results show that protein synthesis was stimulated to a greater extent, chronically than acutely, following CBZ administration at all time points. However, chronically a greater rate of stimulation was observed with free poly $(A)^+$ mRNA than bound poly $(A)^+$ mRNA. Furthermore, the addition of cytosol fraction from control treated brains was unable to stimulate protein synthesis in the CBZ group. Data thus support that the stimulatory property was associated with the polysomal fraction as well as present in the brain cytosol fraction. Whether the stimulation is due to activation of the polypeptide chain initiation reaction of translational processes or because of direct availability of mRNA is now under investigation.

472.18 THE DEVELOPMENT OF GLUTAMINE SYNTHETASE GENE EXPRESSION IN THE RAT CNS, IN VIVO AND IN VITRO. K.M. Mearow and J.F. Mill*. Lab. Mol. Biol., NINCDS, NIH, Bethesda, MD 20892.

Astrocytes are involved in the uptake and regulation of glutamate and GABA in the CNS. A major mechanism for the inactivation of transmitter is a high affinity uptake into the astrocytes. The glutamate taken up in this manner is converted to glutamine, which is then freely available to the adjacent neurons as a transmitter precursor. The enzyme that catalyzes the conversion is glutamine synthetase (GS). Our aim has been to investigate the possible interactions between glial cells and neurons which might be involved in the developmental regulation of this enzyme.

To this end we have cloned a 2.5 Kb rat brain cDNA fragment from a lambda gt11 library through complementarity to a hamster genomic clone of GS. This cDNA clone recognizes a 3 Kb message on northern analysis, which is the approximate size seen in both hamster and avian cells. Partial sequencing of this clone shows a high degree of homology to the published hamster GS cDNA sequence.

Whole brain, brain regions, and various body tissues from rats ranging in age from embryonic day 16 to adult were homogenized in guanidinium isothiocyanate, and the RNA purified by centrifugation through a 5.7 M CsCl step gradient. Equal quantities of total RNA, as determined spectrophotometrically, were run on denaturing formaldehyde gels and transferred to nitrocellulose. Hybridizations were carried out with 32-P labelled nick-translated and cRNA probes to GS. The GS probe detected a similarly sized message in all tissues examined, with the level of GS message being highest in the brain, followed by kidney, liver, skeletal muscle and heart in decreasing order. We have observed the presence of GS message in the brain at embryonic day 16, the earliest day tested. The message level increases from a low level in the early embryo to an elevated amount which is maintained through adulthood.

Since GS has been shown immunohistochemically to be confined to glial cells in the CNS, we have examined the distribution of GS mRNA in cortical and cerebellar astrocytes and cerebellar granule cell neurons cultured in chemically-defined medium. Our results from both northern analysis and in situ hybridization indicate that GS, though present at low levels in neurons, is principally located in astrocytes. Through the use of mixed cultures we can assess the interactions between glial and neuronal cells types that would be expected if astrocytic GS were playing an important role in regulating neurotransmitter glutamate.

MEMBRANE COMPOSITION

473.1 NEURONAL GROWTH-ASSOCIATED PROTEIN 43 IS PRESENT IN PLASMA MEMBRANES OF RAT ASTROCYTES. L. Vitkovic, H. W. Steisslinger*, and M. Mersel*. Lab. Mol. Biol., NINCDS, NIH, Bethesda, MD 20892

One of the neuronal growth-associated proteins, GAP-43, (Mr ca. 43 kDa, pI 4.3) is abundant in growth cone membranes, and corresponds to a major phosphoprotein, pp46, of growth cone-enriched subcellular fraction. One of the two very abundant proteins in plasma membranes isolated from rat brain cortices was recently identified as GAP-43, because of its Mr, pI, in vitro phosphorylation by protein kinase C, and reactivity with anti-GAP-43 antibody (Steisslinger et al., Brain Res., in press).

We now report that this protein appears to be glycosylated because it can be stained with a sensitive silver stain for carbohydrates. Furthermore, a protein of the same Mr and pI as GAP-43, which interacts with anti-GAP-43 antibody on immunoblots, is present in plasma membranes of cultured rat cortical astrocytes. Double immunofluorescence labeling of cells with antibodies against glial fibrillary acidic protein and anti-GAP-43 was observed. GAP-43-like protein was labeled upon lactoperoxidase-catalyzed radioiodination of intact astrocytes suggesting that it is exposed on the cell surface. These observations enlarge the scope of GAP-43's potential functions.

473.2 ISOLATION AND PARTIAL CHARACTERIZATION OF A NON-MYELINATED AXOLEMMA. Mason,P.W., Bigbee,J.W., DeVries,G.H. Depts. of Biochemistry and Molecular Biophysics and Anatomy, Medical College of VA, Virigia Commonwealth Universtiy, Richmond VA.

While numerous studies have shown that the signal inducing glial cells to myelinate resides on the axon, the identity of the factor is unknown. We have previously described the axolemma from myelinated and unmyelinated axons. We are currently isolating and characterizing the axolemma from cerebellar granule cell axons, which are non-myelinated and unensheathed. Comparison of these three axolemmae may identify this myelinating factor and further describe constituents common to axolemma. Granule cells were isolated from the cerebella of 8d rat pups as described by Annunzita et.al. (Dev. Brain Res. 8:261, 1983). The cells were maintained for 8d at which time they have elaborated an extensive neuritic outgrowth. The cells were gently homogenized and the microsomal fraction was separated on a 15%-40% linear sucrose density gradient. Morphological staining on 8d cultures was used in the identification of the perikaryal and neuritic membranes. AChE histochemical reactivity was primarily associated with granule cell perikaryal plasma membranes. Concanavalin A binding combined with immunohistochemical staining was observed predominately along neurites, although some minor binding appeared on the perikaryal membrane. Using a radiometric assay for AChE activity on the fractionated membranes revealed that the perikaryal, AChE enriched, membranes were found in the 25% region of the sucrose gradient. A 3H Con A binding assay revealed the neuritic, con A binding enriched, membranes were located at the 31% sucrose region of the gradient and were also the most enriched in 3H saxitoxin binding. The Con A binding rich membrane fraction isolated by this procedure is enriched in neuritic plasma membrane. Further study of this unique axonal membrane should elucidate some of the signals involved in the complex interactions that occur between axons and glia. (Supported by NS15408 and NS10821)

473.3 PROTEIN KINASE C-DEPENDENT AND -INDEPENDENT CHANGES OF PHOSPHATIDYLCHOLINE BIOSYNTHESIS IN NG108-15 CELLS.
M. Liscovitch* (SPON: E. Hazum). Dept. of Hormone Research, Weizmann Institute of Science, Rehovot, IL-76100, Israel.

Phosphatidylcholine (PtdCho) is quantitatively the most important lipid in mammalian cell membranes. PtdCho was long thought to be a relatively static membrane component subserving a purely structural function. Evidence gathered in recent years suggest, however, that a variety of physiological and pharmacological stimuli can cause rapid changes in PtdCho metabolism. Currently, little is known of the mechanism(s) whereby these changes are effected and their role in cellular responses to external stimuli. We have recently shown that 12-O-tetradecanoylphorbol 13-acetate (TPA), a tumor promoter and activator of protein kinase C, stimulates PtdCho biosynthesis in NG108-15 cells (**Liscovitch et al (1986) J. Neurochem. 46, 1936**). To evaluate the possible involvement of protein kinase C in the regulation of PtdCho metabolism, we examined the action of phospholipase C (which generates endogenous diacylglycerol), as well as exogenous cell-permeant diacylglycerols, on [3H]choline incorporation into PtdCho. Phospholipase C treatment stimulated [^{3}H]choline incorporation dose-dependently at concentrations of 10-100 mU/ml, causing a maximal, 2-3-fold stimulation of the incorporation. However, the effect of phospholipase C was additional to that produced by a maximally effective concentration of TPA (1 μM), suggesting that the two agents may act via different mechanisms. Similar results were obtained when cells were stimulated with sn-1-oleoyl-2-acetylglycerol and sn-1,2-dioctanoylglycerol. In addition, the protein kinase inhibitor H-7 (at 200 μM) partially blocked the action of TPA on [^{3}H]choline incorporation while not affecting that of phospholipase C or diacylglycerol. Pre-incubation of the cells with TPA (0.1 μM) for 24 h reduced protein kinase C activity in the cells and completely abolished the effect of TPA on choline incorporation. In contrast, both phospholipase C- and diacylglycerol-induced stimulation of PtdCho biosynthesis were not inhibited in cells that were desensitized to TPA. These results suggest that diacylglycerol action on PtdCho biosynthesis either is not mediated by protein kinase C activation, or, is mediated by a TPA-*insensitive* iso-enzyme of protein kinase C.

473.4 CHANGES IN PHOSPHATIDYLSERINE CONTENT ALTER PROTEIN KINASE C ACTIVITY IN A HUMAN NEUROBLASTOMA CELL LINE. B.E. Slack*, M. Liscovitch*, J.K. Blusztajn* and R.J. Wurtman. (SPON: G. Adelman) Laboratory of Neuroendocrine Regulation, M.I.T., Cambridge, MA 02139.

Phosphatidylserine (PS), an important, negatively charged phospholipid component of mammalian cell membranes, can potentially influence cell function because of its ability to alter the physical characteristics of lipid bilayers, and to modulate the activity of certain membrane-bound enzymes. We have begun to study the relationship between cell PS composition and function using a human neuroblastoma cell line (LA-N-2) in which it is possible to manipulate phospholipid content. When exposed to liposomal suspensions of PS in serum-free medium for varying periods of time, LA-N-2 cells showed a time and concentration-dependent increase in cell PS content. In the presence of high concentrations (100 μg/ml) of PS in the medium, the PS content of the cell lysate rose within 4 hours, from 5.9 ± 1.2% of total cell phospholipids to 28.3 ± 3.7% (mean ± SEM), and further to 38 ± 1.6% after 24 hours. When lower concentrations of PS were present in the medium, the cell PS content increased progressively over six days (the longest time period studied). Cells treated with PS assumed a rounded configuration, withdrawing the neurites which are normally extended in serum-free medium. Electron micrographs of the PS-treated cells showed vacuoles in the cytoplasm which were absent in controls. These may represent sites of storage for the large amounts of PS in the cells. However, at least some of the PS taken up by the cells was also incorporated into cellular membranes. In preliminary experiments in which cells were lysed and subcellular membrane fractions prepared by centrifugation on a sucrose density gradient, increases of several-fold in the ratio of PS to total phospholipids were measured in some fractions from cells exposed to PS for 24 hours. In parallel experiments, changes in the activity of the PS-dependent enzyme, protein kinase C (PKC) were observed in PS-treated cells. In untreated LA-N-2 cells, PKC was translocated from the cytosol to the membrane in a concentration-dependent manner by the tumor promoting phorbol ester, tetradecanoylphorbol acetate (TPA). This response was accompanied by a rounding of cell morphology similar to that caused by PS. In cells with elevated PS content (28% of total phospholipids) the concentration of TPA required to produce a half-maximal translocation of PKC was increased five-fold, producing a rightward shift in the dose-response curve. These results demonstrate that alterations in membrane PS content in LA-N-2 neuroblastoma cells affect the activity of PKC. This in turn may influence many cell functions known to be regulated by PKC, including *differentiation*, proliferation, and transmitter release.

473.5 MOLECULAR CHARACTERIZATION OF GP50, A NEURON-SPECIFIC, SYNAPTIC-ENRICHED GLYCOPROTEIN. T. Paladino*, P.W. Beesley*, and J.W. Gurd. Dept. of Biochem., Scarborough College, University of Toronto, West Hill, Ontario, Canada M1C 1A4 and Dept. of Biochem., Royal Holloway and Bedford New College, Egham, Surrey TW20 0EX, U.K.

We have previously described a monoclonal antibody (MabSMGP50) which reacts with a major ConA-binding glycoprotein enriched in synaptic membranes (Beesley et al., Brain Res. in press). In the present study we have used MabSMGP50 to investigate the molecular properties and membrane topology of GP50 present in a synaptic membrane fraction prepared from 28-day-old rat forebrains (De Silva et al., Brain Res. 165: 283-293, 1979). The epitope recognized by MabSMGP50 was resistant to digestion with endoglycosidase H and N-glycanase as well as to hydrolysis of acetal linkages with trifluoromethanesulfonic acid (TFMS) or to treatment with alkaline sodium borohydride indicating that the antigenic determinant is not associated with oligosaccharides. Digestion of solubilized, but not membrane-associated, GP50 with Endo H decreased the apparent Mr by 10 to 12 KD. Treatment with either N-glycanase or TFMS caused a similar 10 to 12 KD decrease in Mr suggesting that GP50 does not contain complex N-linked oligosaccharides. The Mr of GP50 was not altered by treatment with alkaline borohydride indicating the absence of O-linked sugar chains. Western blot analysis of synaptic membranes which had been solubilized by SDS in the presence and absence of reducing agent demonstrated that GP50 is not associated with other proteins in the membrane via disulfide linkages. Solubilization of synaptic membranes with SDS followed by digestion with trypsin resulted in the complete loss of the immunoreactive epitope. In contrast when present as a component of intact synaptic membranes, GP50 was relatively resistant to proteolytic digestion indicating that it may be an integral membrane component. Consistent with this interpretation GP50 was not extracted when synaptic membranes were treated with 0.1M Na_2CO_3 pH 11.3. Furthermore following the fractionation of synaptic membrane proteins by temperature-induced phase separation in Triton X-114 GP50 partitioned approximately equally between the detergent and aqueous phases. Anomalous partitioning of integral membrane glycoproteins containing large oligosaccharide moieties into the aqueous phase has previously been reported (e.g. Clemetson et al., BBA 778: 463-469, 1984). These studies indicate that GP50 is an integral membrane glycoprotein and that its oligosaccharides are of the N-linked high mannose and/or hybrid type.
Supported by grants from NSERC and MRC to J.W.G. and a NATO grant for collaborative research (J.W.G. and P.W.B.).

473.6 *PURIFICATION OF A CALSEQUESTRIN-LIKE* PROTEIN FROM BOVINE BRAIN. R.J.J. BENSON & R.E. FINE*. Depts. of Anat. & Biochem., Boston Univ. Sch. of Med., Boston, MA 02118.

In striated muscle the sarcoplasmic reticulum (SR) acts as a calcium-buffering organelle. Its function is to maintain intracellular calcium (Ca^{2+}) at low levels except when an action potential at the plasma membrane stimulates Ca^{2+} release from the SR. This buffering capacity is mediated by the presence of a high-affinity Ca^{2+} and Mg^{2+}-dependent ATPase in the SR membrane; and a low-affinity, high-capacity Ca^{2+} binding protein, calsequestrin (Csq), in the SR lumen.

In neurons, intracellular Ca^{2+} levels also increase episodically due to a voltage-dependent influx of extracellular Ca^{2+}. Removal of this Ca^{2+} is accomplished by sequestration into both mitochondrial and nonmitochondrial stores. Like the SR in muscle, the smooth endoplasmic reticulum (SER) of axons and axon terminals possesses a high affinity Ca^{2+}-Mg^{2+}-dependent ATPase and is able to act as an intracellular sink for Ca^{2+}.

Using methods developed for the isolation of Csq from striated muscle, we have partially purified a protein from bovine brain white matter and from bovine brain synaptosomes which appears to be similar to muscle Csq. This Csq-like protein is removed from microsomal membranes by high pH treatment indicating that it is not an integral membrane protein. When proteins solubilized by high pH are fractionated by hydrophobic chromatography, there is a group of proteins that can be eluted from the column only in the presence of Ca^{2+}. This Ca^{2+}-elutable fraction contains a several proteins which precipitate in the presence of 10 mM Ca^{2+}. Analysis by SDS-PAGE reveals that these three purification steps lead to an enrichment of a doublet in the range of 55 - 60 kD. On western blots a polyclonal antibody raised against chicken cardiac muscle Csq (generously donated by K. Campbell of Univ. of Iowa) labels a doublet in the same molecular weight range. Because white matter and synaptosomes are enriched in this doublet, we believe that a Csq-like protein may play a role in the sequestration of Ca^{2+} in the SER of axons and synaptic endings. Ongoing studies will focus on the subcellular localization of this brain Csq-like protein as well as further characterization of the doublet. This work has been supported by NIH grant, AG05894-14.

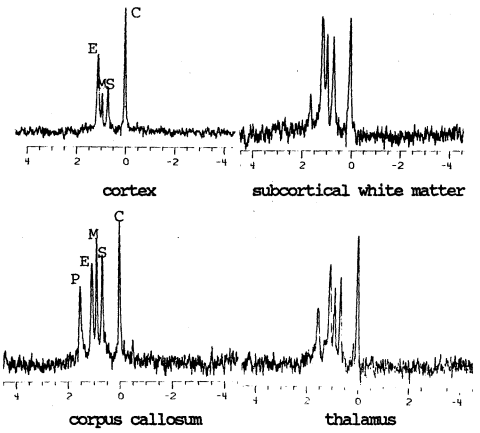
473.7 EFFECTS OF MELATONIN TREATMENT ON PHOSPHOLIPIDS IN THE MEDIAL BASAL HYPOTHALAMUS (MBH) AND CORTEX OF HAMSTERS. K. Rudeen, L. Petterborg, R. Chandrasekhar, M. Navidi and G. Sun. Departments of Anatomy and Biochemistry, University of Missouri School of Medicine, Columbia, Missouri.

Chronic melatonin administration to female hamsters leads to acyclicity and gonadal atrophy. The specific mechanism by which melatonin acts upon the hypothalamic-gonadal axis is unknown. The effects of long term melatonin administration on the incorporation of 32Pi into MBH polyphospho-inisotides and membrane phospholipid composition was examined in this study. Adult female Syrian hamsters were given daily afternoon injections of melatonin (25ug) or ethanol-saline vehicle for 10 weeks. In the first experiment, 12 melatonin-treated and 12 vehicle-treated animals were given 32Pi into the third ventricle either early or late in the light phase of the photoperiodic cycle. The animals were killed 14 hours later and the MBH was excised and processed for incorporation of 32Pi into phospholipids. In the second experiment, 4 melatonin-treated and 4 vehicle-treated hamsters were given 32Pi intraventricularly late in the light phase and killed 14 hours later. Incorporation of 32Pi into polyphosphoinositides (PI's) was measured in the MBH and cortex of the brains. The results from the first experiment indicate the presence of a diurnal rhythm in the incorporation of 32Pi into phospholipids. There was an increase in labelling of PC in animals given the 32Pi late in the photophase compared to those injected with 32Pi early in the daytime. The diurnal variation was more apparent in melatonin-treated animals where 32Pi incorporation into PC and SPH was increased ($p<0.05$) during the nighttime more than during the daytime. Also, aPE and dPE was reduced in the nighttime when compared to daytime levels in melatonin-treated animals. The phospholipid ratios of PS/PI, PS/dPE and PC/dPE were significantly increased ($p<0.05$) during the nighttime in melatonin treated animals. In the second experiment, 32Pi incorporation into polyPI's was significantly increased in the MBH of melatonin-treated hamsters compared to vehicle-treated animals ($p < 0.025$). PIP-2 and PIP were increased in melatonin-treated hamsters, by 15% and 16% respectively. 32Pi incorporation was not significantly altered in the cortex of melatonin-treated hamsters. These data suggest that melatonin may alter membrane phospholipid composition and 32Pi incorporation into polyphosphoinositides. The action of melatonin on MBH phospholipids may be related to a possible mechanism whereby melatonin acts upon the reproductive system by altering phospholipid metabolism in the MBH. (Supported in part by NS20836 to G. Sun and HD17658 to L. Petterborg.)

473.8 REGIONAL PHOSPHOLIPID PROFILE OF THE HUMAN BRAIN DEMONSTRATED BY ^{31}P NMR SPECTROSCOPY. T. Nakada and I. L. Kwee. Neurochem. Res. Lab., VA Med. Ctr., Martinez, CA 94553 and Dept. of Neurology and NMR Facility, Univ. of California, Davis, CA 95616.

Phosphotidyl choline (C), phosphotidyl ethanolamine (E), phosphotidyl serine (S), sphingomyelin (M), and ethanolamine plasmologen (P) represent the five main phospholipids of brain membrane. We have recently shown that the phospholipid profile of brain membrane can be readily studied using ^{31}P nuclear magnetic resonance (NMR) spectroscopy of conventional brain lipid extracts. Although this technique is not directly applicable to live patients, autopsy or biopsy material can be analyzed promptly and accurately.

The following figures are representative ^{31}P spectra of normal cerebral cortex, subcortical white matter, corpus callosum, and thalamus. Unique phospholipid profile consistent with known profiles based on conventional biochemical assays for each region is apparent.



Supported by grants from the NIH (GM 37197, RR 02479) and the Veterans Administration Research Service.

473.9 CHOLESTEROL CONTENT AND FLUID PROPERTIES OF SKELETAL MUSCLE SARCOLEMMAL MEMBRANE VESICLES IN MATURE ADULT AND AGED RATS. K.D. Williams and D.O. Smith. Dept. of Physiology, University of Wisconsin, Madison, WI 53706

In previous research designed to uncover potential changes in membrane lipid composition that could explain age-related increases in permeability and turnover of free choline we evaluated the effects of aging on the composition of skeletal muscle membrane lipids. A rise in plantaris, gastrocnemius, and diaphragm cholesterol levels of about 15% was observed between the ages of 10 and 25 mos in male Fischer 344 rats. Since sarcolemma contains a high fraction of cholesterol, we sought to determine whether this age-related increase was localized in the sarcolemma. A related aim was to establish whether the sarcolemmal fluidity was affected by aging.

Hindlimb muscle (20-30g) was obtained from rats anesthetized with chloral hydrate and homogenized in sucrose buffer. Crude membrane containing sarcolemma from the broken fibers was extracted with LiBr buffer and purified further by washing in KCl buffer and sucrose gradient centrifugation. The sarcolemmal fraction obtained at the interface between 15 and 28.5% (w/v) sucrose did not differ in levels of cholesterol (317 and 332 nmol/mg protein) or phospholipid (879 and 871 nmol/mg protein), or their molar ratio (0.362 and 0.386) between rats aged 10 and 25 mos, respectfully.

To assay membrane fluidity, the activation energies for the sarcolemma ecto-enzyme 5'-nucleotidase were determined from the slopes of Arrhenius plots above and below a characteristic break point at 28°C. However, these properties were similar in sarcolemma isolated from aged and mature-adult rats. The expected effect of elevated membrane cholesterol would have been to increase the activation energy above and to decrease the activation energy below the break point due to a rigidifying and fluidizing effect in the fluid and gel state of the membrane, respectively.

We conclude that the cholesterol content and bulk fluid properties of purified membrane were similar in aged and mature adult rats. Thus, the increased muscle content of cholesterol in aged rats is probably derived from either an extracellular component or is contained in other organelles. In this regard, the cholesterol per mg protein contained in the membrane pelleting to the bottom of the 28.5% sucrose layer was increased by 27% between the ages of 10 and 25 mos. The subcellular source contributing to this rise remains to be determined. Supported by NIH grant AG01572.

474.1 INTRAHIPPOCAMPAL INJECTIONS OF GALLAMINE IMPAIR LEARNING OF A REPRESENTATIONAL MEMORY TASK. W.S. Messer, Jr. and M.D. Miller*. Department of Medicinal Chemistry, College of Pharmacy, University of Toledo, Toledo OH 43606.

Recent studies indicate that gallamine binds selectively to M_2-like muscarinic receptors (Burke, Mol Pharmacol **30:** 58, 1986; Price et al., Biochem Pharmacol **35:** 4171, 1986) which may be presynaptic receptors in forebrain that regulate the release of acetylcholine (Mash et al., Science **228:** 1115, 1985; Schoffelmeer et al., Eur J Pharmacol **128:** 291, 1986). In vitro, gallamine potentiates cholinergic responses including the release of inositol phosphates from brain slices. Intrahippocampal injections of gallamine (> 70 µg bilaterally) produce signs of cholinergic stimulation including salivation, piloerection and tremors in addition to inducing seizures (Messer et al., Fed Proc **46:** 857, 1987).

To determine whether presynaptic cholinergic antagonists can be utilized to potentiate cholinergic activity associated with memory, rats were trained to perform a matching-to-sample memory task in a T-maze. Young male Long-Evans rats were implanted stereotaxically with cannulae aimed at each hippocampus (AP, -4.8; ML, ± 3.7; DV, -4.0). Following recovery from surgery, rats were maintained at 85 % of ad lib weight and trained to move through the maze for food over a period of two weeks prior to learning the memory task.

Once animals responded within three seconds on one-door runs, they were ready for injections and subsequent learning of the win-stay memory task. Animals were pressure-injected with either saline or 10 µg of gallamine triethiodide in 1 µl of saline bilaterally (5 µg to each side) 10 to 30 minutes before each testing session. Animals were given two runs per trial with a 30 second delay between each run. The first (information) run to a single arm was rewarded with food, while the second (choice) run was rewarded only if the animal chose the arm entered previously (win-stay or match-to-sample).

Saline-injected animals (N=4) learned the task slowly and acheived 100 % as a group on the 11^{th} session (block of twelve trials). Gallamine-injected animals (N=5) achieved a higher percentage of correct choices on the first exposure to gallamine, but the difference between the two groups was not significant. By the eighth session, gallamine-injected animals were performing below the level attained by saline-injected animals. Gallamine-injected animals as a group were able to achieve only 74.0 ± 8.7 % correct choices on their best session. The difference between the two groups was statistically significant over the last four sessions ($p < 0.025$ as determined by χ^2 analyses). The data suggest that although gallamine increases acetylcholine release, adaptive mechanisms (such as receptor down-regulation) may prevent an enhancement of memory. This work was supported by a grant from the Ohio Board of Regents.

474.2 SCOPOLAMINE EFFECT ON CHOICE REACTION TIME IN THE MONKEY W. Ashford, J. Bice*. Dept. of Psychiatry, Southern Illinois Univ. Sch. of Med., Springfield, IL 62708.

Cholinergic neurons function in several different brain systems. Those neurons in the nucleus basalis of Meynert, are highly affected in Alzheimer's Disease (AD) while others in the basal ganglia are affected in schizophrenia. The existence of neurons using the same neurotransmitter but anatomically localized in vastly different situations and the complex relationships each neuron has with its own surround have made it difficult to explore the pharmacologic nature of neurons using acetylcholine. Yet, the role of cholinergic drugs is of importance in the treatment of these diseases, and the effects of anti-muscarinic agents have been advocated as their models.

To further elucidate the behavioral effects of anti-muscarinic agents as a potential model of AD, we administered scopolamine to 3 elderly monkeys (>21 years) performing a choice reaction task. Choice reaction is slowed in AD patients. In the task, a red or green TV screen was presented; lever press up or down, respectively, was rewarded with 1 cc grape juice, 320 trials/2 hours. Scopolamine was administered (doses of 10, 20, 15, 5, 25 ug/kg IM) once per week to each monkey (mon, wed, and fri). Inactive placebo (saline) and active placebo (glycopyrrolate: this drug has about twice the peripheral potency of scopolamine in humans, therefore, we used 1/2 scopolamine dose) was given on the other days (drug administered 60 min before testing).

For saline, the average responsiveness was 80% +11%, correctness was 89% +6%. At low doses, scopolamine slightly impaired responsiveness (@5 ug/kg: avg=54%) but not correctness (@5 ug/kg: avg=87%), reaction time was slightly improved (see table). However, at higher doses, scopolamine caused a major decrease of animal responsiveness (@25 ug/kg: avg=25%) and correctness (@25 ug/kg: avg=73%). Only at the highest dose was reaction speed slowed below the saline mean (see table):

	AVERAGE REACTION TIME (msec)				MODE REACTION TIME (msec)			
MONKEY	SALINE	STD	SCOP 5	SCOP 25	SALINE	STD	SCOP 5	SCOP 25
1	717	107	508	817	576	30	505	591
2	1363	119	1201	1797	1207	95	1067	1281
3	1140	120	1028	1543	859	67	748	1124

Glycopyrrolate had no significant or consistent effect on performance, though it had a tendency to impair correctness at the highest doses and delayed effects were suspected.

Low doses of scopolamine may improve motor function by affecting the basal ganglia as such drugs benefit Parkinson patients (a neurological antithesis of schizophrenia). However, the behavioral disruption at the higher doses is similar to that seen with AD patients performing related tasks.

474.3 BACLOFEN POTENTIATES SCOPOLAMINE-INDUCED ALTERATIONS IN RADIAL-ARM MAZE PERFORMANCE OF RATS. Elizabeth S. Sidel, Hugh A. Tilson, Ronnie L. McLamb, H. Scott Swartzwelder and Wilkie A. Wilson. (SPON: Michael Conley).Dept. of Pharmacology, Duke Univ. Med. Ctr. Durham, NC 27710, Dept. of Pharmacology, VA Med. Ctr., Durham, NC 27710, and Lab. Behav. Neurol. Toxicol., NIEHS, NIH, P.O. Box 12233, Research Triangle Park, NC 27709.

Baclofen (β-p-chlorophenyl-GABA) is an anti-spastic drug known for its relative absence of side-effects, even at high doses. It has been suggested that baclofen, when given in combination with drugs having anticholinergic properties, may produce a state of confusion and possible memory deficits greater than those seen with anticholinergic drugs alone. To test this hypothesis, 16 male Fischer-344N rats were trained on an eight-arm radial maze for food reinforcement. The effects of various doses of baclofen (1.25 or 2.5 mg/kg) and scopolamine (0.1875, 0.375, and 0.75 mg/kg), alone and in combination were determined. Baclofen alone did not significantly affect measures of cognitive function (number of correct responses in the first eight responses, total errors), or the time required to complete the maze. Scopolamine alone decreased the number of correct responses in the first eight arm choices, while increasing both the number of errors and the time necessary to complete the maze. A subjectively-noted increase in circling behavior and vacillatory responses was also present in scopolamine-treated rats. When the two drugs were co-administered, baclofen had no effect on the number of errors or time required to complete the maze in the presence of scopolamine; however, in conjuction with the high dose of scopolamine, it significantly potentiated the decrease in number of correct responses in the first eight arm choices. In a subsequent experiment, the interactive effects of baclofen and scopolamine on motor activity were examined. Baclofen exhibited no dose-response effect on motor activity, whereas all doses of scopolamine increased activity. The higher dose of baclofen attenuated scopolamine-induced hypermotility by 50%, but the lower dose of baclofen was not effective. These data support the conclusion that baclofen exacerbates behavioral deficits produced by drugs with anticholinergic properties in the radial-arm maze, yet antagonizes the hypermotility seen with these drugs in motor activity chambers. (This work supported in part by NSF grant RCD8651791.)

474.4 EFFECTS OF THE ANTICHOLINESTERASE PARAOXON AND THE MUSCARINIC ANTAGONIST ATROPINE ON RETENTION OF LEARNED BEHAVIOR AND ON STRIATAL MUSCARINIC RECEPTORS. J.E. Chambers and H.W. Chambers*. Depts. of Biological Sciences and Entomology, Mississippi State University, Mississippi State, MS 39762.

Cholinergic hyperactivity and hypoactivity would be induced following administration of an acute treatment of an anticholinesterase or an acetylcholine antagonist, respectively. In the case of a persistent organophosphate anticholinesterase, the neurochemical lesion lasts a number of days following treatment. Acute high level exposures to paraoxon (P; diethyl p-nitrophenyl phosphate, the active metabolite of the insecticide parathion), and two forms of atropine (atropine sulfate, AS, active centrally; and atropine methyl bromide, AMB, inactive centrally) alone or in combination, were studied for their effects on the retention of fixed ratio performance and on muscarinic receptors in the corpus striatum of male rats. High doses were given to mimic a severe accidental poisoning. Adult male rats (Rattus norvegicus), Sprague Dawley derived strain, which were food deprived, were trained to an FR10 schedule of bar pressing for food pellet reinforcement. Following stabilization, they were injected intraperitioneally with the drugs or vehicle and were tested for retention of FR10 performance for up to 4 days following treatment. Animals were sacrificed at 1,2 or 4 days following treatment for assay of ^{3}H-quinuclidinyl benzilate binding to muscarinic receptors and for acetylcholinesterase activity. Performance was greatly depressed by AMB, AMB+P and AS+P 1 day after treatment with a gradual recovery. A slight decrease in average performance was observed after exposure to P alone at 1 day with recovery by 2 days. Performance was unaffected by AS. The percent inhibition of brain acetylcholinesterase observed at 1,2 and 4 days after treatment with P was about 85%, 65% and 45%, respectively. Small increases in muscarinic receptor numbers were observed 1 day after treatment with atropine and decreases were observed 4 days after treatment with P. The changes in fixed ratio performance following drug treatment are considerably more dramatic than the changes observed in striatal muscarinic receptors. (Supported by EPA grant R-811295).

474.5 EFFECTS OF NEUROACTIVE AGENTS ADMINISTERED INTRACEREBRALLY ON BEHAVIORAL INHIBITION IN CATS. M.M. Curtis*, P.M. Saxton (Wixom) and J. Siegel. Institute of Neuroscience and School of Life and Health Sciences, Univ. Delaware, Newark, DE 19716.

In a series of experiments, CNS inhibitory mechanisms were investigated using a differential reinforcement of low rate of response (DRL) operant task. Six cats were trained on the DRL task which involves an active behavioral inhibitory process. Cannulae were implanted in the basolateral amygdaloid and medial septal nuclei for the administration of serotonin (5-HT)(45 μg), fluoxetine (16 μg), L-norepinephrine bitartrate (NE)(40 μg), or acetylcholine (ACh)(40 μg). All drugs were administered in a 1.0 μl suspension. Control conditions involved injections of vehicle and performance on a noninhibitory task (a fixed ratio schedule).

Performance indices (errors, reinforcements, and inter-response times [IRTs]) were tabulated for each 5 min period in a 30 min test session. Errors on the DRL schedule were made when an animal failed to withhold a bar-press for a specified period of delay time (e.g., 15 sec). Reinforcement (liquid mash) was received when an animal bar-pressed at the correct time.

Fluoxetine, which elevates serotonin levels, improved performance on the DRL (errors decreased; reinforcements increased) when injected into the amygdala and septum. 5-HT into both brain sites reduced errors and reinforcements through an overall slowing in bar-press response rates (IRTs increased). Both NE and ACh impaired DRL performance (errors increased; reinforcements decreased) when injected into the septum. Both NE and ACh into the amygdala caused a slowing in response rates.

These preliminary results indicate that there is a complex interaction of several neurotransmitter systems (5-HT, NE, and ACh) in the amygdala that appears to exert a facilitatory influence on a behavioral inhibitory system. CNS inhibitory mechanisms are greatly impaired when NE levels are elevated in the septum. Other agents (e.g., clonidine) will be tested prior to histological verification of cannulae placement.

474.6 GLUCOSE AND EPINEPHRINE ATTENUATION OF SCOPOLAMINE-INDUCED LOCOMOTOR ACTIVITY IN MICE. W.S. Stone, K.L. Cottrill* and P.E. Gold. Dept. Psychology, University of Virginia, Charlottesville, VA 22903.

Many species exhibit impaired cholinergic function in old age, with prominent deterioration of cholinergic systems observed in various age-related disorders, including Alzheimer's Disease (AD). In addition, the presence of age-related declines in memory storage have also been associated with both normal and pathological aging processes. We have previously observed that epinephrine (EPI), which liberates hepatic glucose (GLU) among its other physiological activities, improves memory in aged rats and mice (Sternberg et al., Beh. Neur. Biol., 1986). Scopolamine (SCOP)-induced amnesia has been used as a model of age-related memory impairment. Recently, we found that SCOP-induced amnesia can be partially attenuated by posttraining (EPI) and (GLU), but not arecoline (AREC), injections in young mice. The dose-response curve was an inverted-U with maximal attenuation at 0.05 mg/kg with EPI and 100 mg/kg with GLU. In the present experiment, we explored the possibility that hyperactivity produced by SCOP could also be attenuated by EPI or GLU injections.

Activity levels, measured by the number of lines crossed in an acrylic compartment, were assessed for 10 minutes, starting 50 minutes after male mice (DUB-ICR) were injected with SCOP (3.0 mg/kg), meth-SCOP (3.0 mg/kg) or saline (SAL). Only SCOP increased activity levels above those of SAL, suggesting that the effect was produced centrally rather than peripherally. Additional groups of mice were then injected with EPI, GLU, AREC, or physostigmine (PHYSO), administered 30 min after the SCOP (20 minutes before assessing activity levels). The results indicate both EPI and GLU significantly attenuated SCOP induced activity in an inverted-U dose dependent manner, with maximally effective doses comparable to those observed in memory tests. Of the cholinergic agonists, PHYSO (0.2 mg/kg) but not AREC (10 mg/kg) significantly attenuated the hyperactivity. In addition, a combination of GLU and PHYSO produced significantly greater attenuation than did either drug alone.

These results indicate that EPI and GLU can reduce the effects of cholinergic blockade induced by SCOP as measured behaviorally using either activity or memory tests. They also suggest that GLU may interact synergistically with cholinergic agonists to attenuate effects of cholinergic impairments seen during aging. The attenuation of SCOP impairment by non-cholinergic agents suggests the importance of interacting neural systems for an understanding of both age-related changes and their possible amelioration. [Supported by the Office of Naval Research (N00014-85-K0472) and the American Diabetes Association.]

474.7 **DISCRIMINATIVE STIMULUS PROPERTIES OF PHYSOSTIGMINE IN RATS.** S.R.Franklin* and A.H.Tang, CNS Research, The Upjohn Company, Kalamazoo, MI 49001

The cholinesterase inhibitor, physostigmine (Phy), has been shown to improve memory functions in both normal human subjects and in Alzheimer's patients (Davis et al., Science 201:272, 1978; Davis and Mohs, Am. J. Psychiatry 139:1421, 1982). Experimental subjects have reported characteristic subjective effects (e.g. nausea, slowed thoughts, mild sedation, etc.) produced by Phy (Davis et al., Psychopharmacol. 51:23, 1976). In this study, Sprague-Dawley rats were trained to discriminate a subcutaneous injection of Phy (0.2 mg/kg) from a similar injection of saline in a two-lever, food-reinforced behaviorial paradigm. This training dose of Phy reduced the response rate to about 50% of that in saline sessions. The discriminative stimulus (DS) effect of Phy at the training dose is mediated by a muscarinic mechanism in the CNS, since it was antagonized by scopolamine (0.1-0.3 mg/kg), but was unaffected by methyl-scopolamine (1 mg/kg) or pirenzepine (3 mg/kg), which do not enter the brain readily. The rate-reducing effect of Phy was not affected by the above antagonists. The peripherally-active cholinesterase inhibitor, neostigmine, produced predominantly saline-appropriate lever choice at doses that did not completely suppress responding. Compounds which produced averages of greater than 80% responses on the Phy lever are: Compound BM-5 (N-methyl-N-(1-methyl-4-pyrrolindino-2-butynyl)-acetamide), THA (1,2,3,4-tetrahydro-9-aminoacridine), RS-86 (2-ethyl-8-methyl-2,8-diazaspiro-(4,5)-decan-1,3-dion hydrobromide), and pilocarpine. In comparison, oxotremorine, arecoline, and aceclidine (3-acetoxyquinuclidine) produced a maximum average responding of 40-60% on the Phy lever before the behavior was completely abolished. The DS effect of Phy in rats may be related preferentially to activation of the M1 muscarinic receptor in brain.

474.8 BEHAVIORAL AND MORPHOLOGICAL STUDIES OF THE INTERACTION BETWEEN EXERCISE AND PHYSOSTIGMINE. S.B. McMaster and R.E. Foster. Pathophysiology Division, U.S. Army Medical Research Institute of Chemical Defense, APG, MD 21010-5425.

Exercise has been shown to influence behavioral sensitivity to a number of compounds acting primarily on the cholinergic system. Acute exercise appears to increase sensitivity. Conversely, a decrease in sensitivity can be demonstrated in response to chronic exercise. The effects of acute and chronic exercise on behavioral sensitivity to physostigmine (PHY), as well as the effect of acutely administered PHY on ability to exercise, have been evaluated in rats. Correlative morphological examination of muscle tissue from drug-challenged, exercised animals has been completed.

Experiment 1. Rats trained to lever press for food reward under a fixed ratio (FR30) schedule of reinforcement completed a 10-week exercise conditioning program. Non-exercise conditioned rats, trained on the FR30 task, served as control subjects. When rested, the control animals displayed a typical dose-related performance decrement (15, 46, 73 and 69%) in response to four doses of PHY (50, 100, 150 and 200 ug/kg). Rats that had completed the exercise-conditioning program continued to perform at near-baseline levels over the same dose range. When a 15-min treadmill session preceeded PHY administration, FR30 performance remained above 60% of baseline levels in the exercise-conditioned group across all doses. In contrast, responding by control animals fell to below 20% of baseline performance at the lowest dose.

Experiment 2. Administration of PHY prior to exercise was studied in an additional group of exercise-conditioned rats. The higher dose studied, 75 ug/kg, is below the threshold for FR30 behavioral effects, produces 66% cholinesterase (ChE) inhibition in whole blood, and does not produce neuromuscular lesions in sedentary rats. When administered 15 min prior to exercise, this dose prevented completion of a standard exercise session. The lower dose studied, 7.5 ug/kg, produced 30% inhibition of whole blood ChE and no apparent difficulty in completion of a 30-min exercise session. Twenty-four hours after drug administration, animals were anesthetized with sodium pentobarbitol and vascularly perfused with saline followed by gluteraldehyde fixative. Diaphragm, soleus and extensor digitorum longus muscles were dissected and processed for histological localization of ChE at neuromuscular junctions (NMJs). Small pieces of muscle containing stained NMJs were further processed for standard electron microscopy. Analysis of thin sections from the high dose group revealed occasional myofibrillar damage but little evidence of extensive NMJ damage.

474.9 EFFECTS OF NICOTINE AND CANNABINOIDS ON EXTRAPYRAMIDAL MOTOR BEHAVIORS. P.Z. Manderscheid, D.E. Moss, H. Kobayashi*, and S.P. Montgomery*. Lab. of Psychobiochemistry, Dept. of Psychology, Univ. of Texas at El Paso, El Paso, TX 79968.

Although delta-9-tetrahydrocannabinol (THC) and related cannabinoids have been studied extensively for several years, the biochemical mechanism(s) by which THC alters physiological and behavioral functions is still unknown. The working hypothesis of our laboratory is that the cannabinoids are acting as nicotinic cholinergic agonists within the extrapyramidal system to potentiate hypokinesia induced by direct or indirect dopamine antagonists.

The conditions under which extrapyramidal effects of cannabinoids and nicotine can be observed are relatively specific: the most important being the blockage of dopaminergic function within the striatum (e.g., after reserpine (RES)-, fluphenazine HCl (FLU)-, or haloperidol-induced hypokinesia)(Moss et al., 1984).

Our nicotinic hypothesis of cannabinoid action has been derived from experiments which show that (1) THC or a combination of THC and RES does not affect the depletion of dopamine in the striatum (Moss et al., 1984) and that, (2) THC/RES hypokinesia is completely reversed by ethopropazine, an anticholinergic-antiparkinsonian agent (Moss et al., 1984).

Montgomery et al. (1985) have shown that intracranial injections of (-)-nicotine into the caudate-putamen (CP) of reserpinized rats potentiate hypokinesia (as measured by the bar test) in a manner exactly parallel to levonantradol, a synthetic analog of THC. In a test of the nicotinic hypothesis, mecamylamine (MEC), a non-quaternary nicotinic cholinergic antagonist, when injected into the CP of reserpinized rats prior to nicotine, blocked nicotine-potentiated hypokinesia. More importantly, however, with regard to cannabinoid action, systemic injections of MEC (1.0 mg/kg) into FLU pretreated rats (0.1 mg/kg i.p.) is effective in blocking THC (10 mg/kg p.o.)-potentiated hypokinesia. An unexpected finding in this experiment was that MEC alone also produced a mild hypokinesia in the FLU pretreated rats. We believe that this is an artifact that can be eliminated by using a lower dose of MEC.

If the nicotinic hypothesis is correct, other pharmacologically consistent interactions with known nicotinic agonists and antagonists are expected. However, showing that nicotine can mimic the behavioral effects of THC is not sufficient to prove that the same neurophysiological substrate is involved. In order to prove that cannabinoids are working as nicotinic agonists, receptor binding experiments need to be conducted to determine if the behavioral results obtained with nicotine and the cannabinoids can be explained by a direct action on central nervous system nicotinic receptors. If our nicotinic hypothesis of cannabinoid action is correct, it must be recognized that this may still be only one of several possible cannabinoid actions.

474.10 A STRAIN COMPARISON OF NICOTINE SENSITIVITY IN ADRENALECTOMIZED MICE. J.R. Pauly, E.A. Ullman*, and A.C. Collins. Institute for Behavioral Genetics. University of Colorado, Boulder, CO 80309.

A major focus of our laboratory is the identification of genetic factors that regulate behavioral and physiological responses to acute nicotine administration. We have demonstrated that intraperitoneal injections of nicotine stimulate a dose-dependent release of corticosterone (CCS) in the mouse and that this process is regulated by genotype. If the CCS that is released in response to nicotine injections has a role in the mediation of nicotine's actions, then animals that have altered adrenal status should also have altered sensitivity to nicotine injections. This hypothesis was tested by treating adrenalectomized (ADX) mice of four inbred strains with nicotine and measuring behavioral and physiological responses in a test battery.

Adult mice (60-90 days) of four strains (BUB, C3H, C57, and DBA) were ADX under pentobarbital anesthesia (54 mg/kg). Following surgery, animals were housed individually and provided with 0.9% saline in their drinking water. After one week of recovery, nicotine (0.25-1.5 mg/kg) or saline was injected intraperitoneally and the animals were tested for Y-maze activity (crosses and rears), acoustic startle response, heart rate, and body temperature. Nicotine elicited a dose-dependent increase in startle response and a decrease in all other parameters. ADX mice of the C3H strain were significantly ($p < .05$) more sensitive than sham operated controls on all parameters tested. In DBA mice, ADX rendered the animals more sensitive to nicotine's effects on only heart rate and body temperature. ADX mice of the C57 (heart rate) and BUB (startle response) strains were more sensitive than controls for only one test. ADX could induce enhanced sensitivity to nicotine as a result of changes in nicotine metabolism or as a result of tissue sensitivity changes. Using biochemical receptor binding assays we have begun to investigate the effects of ADX on brain nicotinic receptors. Preliminary data suggest an increase in brain α-bungarotoxin binding sites in several brain regions of C57 mice. These studies may help explain the genetic regulation of nicotine's actions on the mouse central nervous system. Supported by NIMH training grant MH-16880 and a grant from R.J. Reynolds, Inc.

474.11 A DOSE-RESPONSE CURVE FOR NICOTINE-INDUCED FLAVOR AVERSION LEARNING IN RATS. T.A. Landrum*, D.G. Gilbert*, and R.A. Jensen. Biopsychology Laboratory, Department of Psychology, Southern Illinois University, Carbondale, IL 62901.

The release of opioid neuromodulators in response to nicotine administration has been suggested as an underlying reinforcing mechanism for tobacco use (Pomerleau, O.F. & Pomerleau, C.S., Neurosci. & Biobehav. Rev., 8:503, 1984). Studies providing evidence in support of this hypothesis have been performed in animal and human subjects. However, some of these studies may have employed inappropriately high doses of nicotine -- doses which may induce toxicosis. Such nicotine-induced toxicosis may cause a generalized stress response which is associated with the release of opioid hormones. Therefore, it is important to determine a critical dose of nicotine above which possible confounding toxicosis is produced. One method of determining whether a given dose of a compound results in malaise in rats is through the use of taste-aversion-learning techniques.

Male Sprague-Dawley rats were maintained on a 20-min-per-day schedule of water deprivation for 5 days prior to training on a taste-aversion learning task. Using a two-bottle procedure, the rats were given a 10-min presentation of a 0.1% saccharin solution on the training day. Ten minutes after drinking was completed, animals received (i.p.) one of six logarithmically spaced doses of nicotine (Sigma) (0.01, 0.022, 0.046, 0.10, 0.22, 0.46 mg/kg), saline, or 2.0 ml of 0.47 molar lithium chloride. On the test day, 72 hours after conditioning, each rat was given a preference test in which one bottle contained the 0.1% saccharin solution while the other contained distilled water.

Significant reductions in the amount of saccharin consumed, in relation to animals that received saline injections, were seen in animals that had been injected with nicotine above the 0.022 mg/kg dose. The 0.022 and 0.01 mg/kg doses of nicotine produced no suppression of saccharin consumption. These results suggest that the critical dose for nicotine-induced toxicosis lies between 0.022 and 0.046 mg/kg. In a previous study (Iwamoto, E.T. & Williamson, E.C., Pharm. Biochem. & Behav., 21:527, 1984) a significant reduction in saccharin consumption was seen with a nicotine dose of 0.5 mg/kg and a non-significant reduction at a dose of 0.16 mg/kg. The present results suggest that nicotine can serve as an effective UCS in a taste-aversion paradigm at doses substantially lower than those previously reported. Studies reporting specific nicotine effects at higher doses may actually be measuring nonspecific results of nicotine toxicosis.

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474.12 ACUTE AND CHRONIC EFFECTS OF NICOTINE ON RAT STRIATAL NICOTINIC AND DOPAMINERGIC SYSTEMS. Y.K. Fung and Y.S. Lau. Dept. of Oral Biology, University of Nebraska Medical Center, College of Dentistry Lincoln, NE 68583 and Dept. of Pharmacology, School of Medicine, Creighton University, Omaha, NE 68178.

Some of the central effects of nicotine are suggested to be mediated via the nicotinic receptors located in the corpus striatum. In most studies high doses of nicotine were administered to animals so that large amounts of nicotine would be expected to accumulate in the central nervous system instantaneously. In the present study, nicotine (1.5 mg/kg/day) was administered to rats by the subcutaneous implantation of Alzet osmotic mini-pumps for 1, 5 and 14 days. This dose and method of continuous nicotine administration were chosen in an attempt to approximate the dosage pattern of an individual who smokes one to two packages of cigarettes a day. Control animals were implanted with osmotic mini-pumps containing physiological saline.

One day pretreatment of animals with nicotine did not alter the characteristics of ^{3}H-nicotine and ^{3}H-spiperone binding to striatal nicotinic and dopaminergic binding sites. This nicotine pretreatment did not affect the apomorphine (0.7 mg/kg, s.c.) or nicotine (0.2 or 0.4 mg/kg, s.c.)-stimulated locomotor activity. However, the (+)-amphetamine (1 mg/kg, s.c.)-induced hyperactivity was attenuated. These results suggest that nicotine acutely inhibits the presynaptic action of (+)-amphetamine without directly affecting the stimulatory responses of either nicotinic or dopaminergic receptors.

In animals pretreated with nicotine for 5 days, we observed an increase of nicotine (0.2 or 0.4 mg/kg, s.c.)-induced hyperactivity, which was associated with an increase of striatal nicotinic receptors. The striatal dopaminergic receptor binding sites were not changed. In animals pretreated for 14 days with nicotine, the nicotine-induced locomotor response remained to be potentiated. This response was correlated with an elevation of striatal dopaminergic receptors, whereas the striatal nicotinic receptors had returned to normal. These results indicate that chronic administration of nicotine produces behavioral hyperactivity in the rats by two mechanisms which are time-dependent. Repeated administration of nicotine initially causes an up-regulation of nicotinic receptors and subsequently leads to the development of dopaminergic receptor supersensitivity in the corpus striatum.

This study was supported by a grant from Smokeless Tobacco Research Council, Inc., N.Y.

474.13 EFFECTS OF CARBAMATE PRETREATMENT AND OXIME THERAPY ON SOMAN-INDUCED PERFORMANCE DECREMENTS AND BLOOD CHOLINESTERASE ACTIVITY IN PRIMATES. D. W. Blick*, M. R. Murphy*, G. C. Brown*, S. L. Hartgraves and M. G. Yochmowitz*. USAF Sch. Aerosp. Med., Radiat. Sci. Div., Brooks AFB, TX 78235

Performance by well-trained Rhesus monkeys of the Primate Equilibrium Platform (PEP) compensatory tracking task was measured for 30 min before and 90 min after exposure to soman (2.05-2.78 μg/kg) with simultaneous atropine (97 μg/kg) therapy. The ED50 soman dose to produce a PEP performance decrement estimated by the up-and-down method was 2.27 μg/kg (95% confidence interval: 2.15 $\leq$ ED50 $\leq$ 2.40 μg/kg). Another group of monkeys was pretreated with 150 μg/kg of pyridostigmine bromide (PYR) 30 min before soman exposure and given a therapeutic dose (17 mg/kg) of 2-PAM Cloride (2-PAM) in addition to atropine. This group had an ED50 soman dose of 2.58 μg/kg (95% confidence interval: 2.41 $\leq$ ED50 $\leq$ 2.76 μg/kg).

A similar pretreatment-therapy regimen provides substantial protection against the lethal effects of soman exposure. However, the protection against soman-induced performance decrements that we measured (Protection Ratio = 1.14) has little or no practical significance.

The control group and the group treated with PYR and 2-PAM had similar levels of serum cholinesterase (ChE) inhibition 90 min after soman exposure, but the treated animals had significantly lower levels of ChE inhibition 24 h and one week later. A separate study of changes in ChE activity after soman exposure showed that 2-PAM therapy reduced ChE inhibition at all times after soman exposure. PYR pretreatment changed the time-course ChE inhibition, producing higher levels of inhibition early ($\leq$3 h) and lower levels later ($\geq$6 h) after soman exposure. The 2-PAM and PYR effects combined additively.

Results for inhibition of ChE activity in whole blood were qualitatively similar, except that ChE inhibition in PYR pretreated animals never exceeded the inhibition observed in animals that did not receive PYR pretreatment.

474.14 EFFECTS OF PHYSOSTIGMINE ON CEREBRAL GLUCOSE METABOLISM (CMR_{glc}) MEASURED BY ^{18}FDG/PET STUDIES IN NORMALS. W.W. Barker*, D. Loewenstein*, J.Y. Chang*, F. Yoshii*, A. Apicella*, P. Kothari* and R. Duara. (SPON: K. Gross-Glenn) Mount Sinai Medical Center, Miami Beach, FL 33140.

Physostigmine, a short-acting cholinesterase inhibitor, improves memory in normal subjects (Davis, KL, et al, Science 201:272-274, 1978) and in Alzheimer's patients (Christie, JE, et al, Brit J Psychiat 138:46-50, 1981) at I.V. doses of 0.005 to 0.01 mg/Kg. The cerebral metabolic effects of physostigmine were tested in four normal subjects (2 male, 2 female; $\bar{x}$ age:42.5 $\pm$ 15 y) using a 15 min infusion of 0.01 mg/Kg physostigmine in 50 ml saline administered inbetween two consecutive PET studies. Subjects performed a complex reading memory task (CRMT) continuously for 30 min after the injection of ^{18}FDG and were scanned for 20 min. Physostigmine infusion was started at the end of the first scan and completed 5 min. after the second ^{18}FDG injection. After the second injection was given CRMT was performed again for 30 min and followed by another 20 min scan. CMRglc for the second scan was calculated using a double-injection model validated by us (Chang, et al, J Nucl Med, In Press). CMRglc (mg/100g/min) was 8.91 $\pm$ 1.48 before and 8.89 $\pm$ 1.16 after physostigmine. No specific regional effects were found. CRMT scores did not change (53.5% correct before and 53.9% correct after physostigmine). These results agree with the observations in rats where physostigmine had no overall effect on CMRglc, but did produce highly localized activation in the superior colliculus and anteroventral thalamus (Dietrich, et al, Ann Neurol 16:117-118, 1984). These structures are well below the resolution of the PETT V system used. These studies set the stage for similar investigations in Alzheimer's patients.

474.15 DOSE-DEPENDENT EFFECTS OF INTRADENTATE COLCHICINE ON BEHAVIOR AND RESPONSIVENESS TO SCOPOLAMINE. N. John Peterson* and Hugh A. Tilson. (SPON: R. Rech). Lab. Behav. Neurol. Toxicol., NIEHS, NIH, Research Triangle Park, NC 27709.

The purpose of these experiments was to determine the dose-dependent alterations in behavior and hippocampal morphology that follow intradentate administration of colchicine in rats. Male, adult Fischer-344 rats received bilateral injections of either 0, 1.25 or 2.5 ug of colchicine into the dorsal and ventral regions of the dentate gyrus. Three weeks later, motor activity of rats was measured over a period of 60 min on three consecutive days. Colchicine produced a dose-dependent increase in both rearing and horizontally directed ambulation. Colchicine-treated rats differed from controls more on the last day of testing than the first day of testing. Light microscopic examination of hippocampal morphology indicated that colchicine produced significant decreases in the length and width of the granule cell lines; the dorsal region of the hippocampus was affected more than the ventral region. Decreases in pyramidal cell length were evident (10-15%), but were generally not dose-dependent. The widths of the CA1 and CA3 regions of the hippocampus were not affected by colchicine and there was no apparant differential sensitivity between the dorsal and ventral hippocampus. Rearing and ambulatory motor activity were statistically correlated with granule cell length and width, particularly for the last day of testing.

Another group of rats was given various doses of colchicine and three weeks later, injected with isotonic saline, s.c., and motor activity tested for 60 min. One week later, the rats were challenged with 0.75 mg/kg of scopolamine given s.c. There were dose-related increases in motor activity in rats having received colchicine after either saline or scopolamine. However, colchicine-treated rats appeared to be less sensitive to the stimulatory effects of scopolamine than controls. These data are consistent with previous results showing a down-regulation of cholinergic muscarinic receptors in the hippocampus of colchicine-treated animals.

In summary, intradentate administration of colchicine produced a dose-dependent loss of granule cells in the hippocampus, which was highly correlated with increased motor activity and alterations in sensitivity to scopolamine. Colchicine appears to be a useful agent to study the functional role of the dentate gyrus and compensatory changes in the hippocampus following lesioning.

474.16 INTRACRANIAL INJECTIONS OF ACETYLCHOLINE AFFECT BEHAVIORAL THERMOREGULATION IN GOLDFISH. L.P. Wollmuth, L.I. Crawshaw*, and R.N. Rausch*. Dept. of Biology, Portland State Univ., Portland, OR. 97207.

Cannulae were implanted into forebrain loci of goldfish to determine the effects and site of action of intracranial acetylcholine (ACh) injections on behavioral thermoregulation in goldfish (Carassius auratus; 40-80 gms). Temperature selection was monitored in an aquatic thermal gradient. Positions of fish in this gradient were imaged using a wide angle camera located above the gradient and digitized by an Occulus frame grabber. After an experiment, the gradient was calibrated, and this information was used to convert the stored position values into temperature values. Following 30 min in a thermal gradient, implanted fish were injected with acetylcholine chloride (2-50 ug ACh) in a total volume of 0.2 ul (0.7% NaCl was used as a vehicle). Injections of 10, 25, and 50 ug ACh into the most anterior aspect of the nucleus preopticus periventricularis (NPP; Peter, R.E. & V.E. Gill, J. Comp. Neurol., 159:69, 1975) led to consistent, dose-dependent decreases in selected temperature (T_{sel}). Inconsistent decreases in T_{sel} were observed following injections of 5 ug ACh into the same neuroanatomical site. No effect on T_{sel} was observed following injections of 2 ug ACh or control injections. At sites removed from the anterior NPP, no effect on T_{sel} was observed following injections of 25 or 50 ug ACh. Other studies in this laboratory have demonstrated that ethanol and norepinephrine (NE) have thermoregulatory effects when injected into the anterior brainstem. The site of action of ACh was contiguous with that of ethanol and anterior to that of NE. We conclude the cholinergic inputs to the anterior NPP lead to a decrease in the regulated temperature in goldfish.

475.1 EFFECTS OF AMANTADINE ON INTRAVENOUS COCAINE SELF-ADMINISTRATION IN RATS. S.C. Cunningham*, S.I. Dworkin, J.E. Smith and N.E. Goeders (SPON: R.D. Brown). Departments of Pharmacology and Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

The ability of amantadine to release dopamine from extragranular storage sites (Farnebo et al, Eur J Pharm 16:27, 1971) is believed to be the mechanism for the anti-parkinsonian actions of the drug. This mechanism is similar to that of psychomotor stimulants such as amphetamine and cocaine which also facilitate dopaminergic neurotransmission. In clinical trials with cocaine-dependent subjects, it has been reported that amantadine was effective in reducing cocaine craving while producing no side effects (Tennant and Sagherian, Arch Intern Med 147:109, 1987). Since intravenous self-administration is considered to be an animal model of drug abuse, this study was initiated to determine the effects of amantadine on cocaine self-administration in rats.

Male Fisher rats were implanted with chronic jugular catheters and trained on a fixed-interval 300 sec (FI 300) schedule of cocaine reinforcement (0.2 ml infusion of 0.5 mg/kg cocaine hydrochloride in isotonic saline). Animals were run 5 days a week for 2.5 hr a day. Amantadine was administered in three doses (10, 20, and 30 mg/kg, ip) 15 min prior to the session 2 days a week.

Amantadine pretreatment resulted in no significant effects on the total number of infusions. However, at 10 mg/kg the overall response rate was reduced to 49.6% of the vehicle rate. At 20 mg/kg the rate decreased to 66.3% (SEM = ± 23.0%) of vehicle. The relatively high variability of the effects of the drug on response rates was likely due to a marked attenuation of the rate decreasing effects of amantadine with subsequent testing, suggesting an acute tolerance to the rate decreasing effects. At 30 mg/kg, response rate was reduced to 50.6% of vehicle responding.

The predominant effect of amantadine appears to be a decrease in the overall rate of cocaine maintained responding at each dose tested. This reduction in response rate is similar to that found by increasing the dose of cocaine, suggesting that amantadine had an additive effect with the rate decreasing effects of cocaine. Since the total number of infusions per session was not affected, amantadine does not appear to directly influence the reinforcing properties of cocaine, although this effect cannot be ruled out. If ratio schedules of cocaine self-administration had been investigated, the amantadine-induced decrease in the response rates would have likely resulted in a decrease in infusions per session. Further determinations of the effects of amantadine on cocaine self-administration are currently in progress.

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475.2 VENTRAL PALLIDAL LESIONS PRODUCE DECREASES IN COCAINE AND HEROIN SELF-ADMINISTRATION IN THE RAT. C.B. Hubner* and G.F. Koob. Scripps Clinic and Research Foundation, La Jolla, CA. 92037.

Using the intravenous drug self-administration paradigm, previous work in our laboratory has led to the hypothesis that separate neural systems mediate the reinforcing properties of cocaine and heroin and, furthermore, that these pathways appear to converge at the level of the nucleus accumbens. The results from these studies indicated that presynaptic dopamine terminals within the region of the nucleus accumbens are important for cocaine reward and that opiate receptors postsynaptic to the mesolimbic dopamine system within the nucleus accumbens play a critical role in mediating heroin reward. The ventral pallidum (substantia innominata/lateral preoptic area) is one of the major recipients of nucleus accumbens efferent fibers. The purpose of the present study was to determine the importance of this nucleus accumbens-ventral pallidum pathway in mediating cocaine and heroin reinforcement in the rat.

12 Wistar rats were trained to self-administer intravenous cocaine (0.75 mg/kg/inj) in 3 hour daily sessions on an FR 5 schedule. After reaching steady baseline responding (± 10% of the average for 3 consecutive days) rats received either bilateral injections of vehicle (N=6) or ibotenic acid (N=6) into the ventral pallidum. When tested 5 days post-lesion, subjects in the lesion group showed a significant decrease in cocaine self-administration when compared to vehicle control animals. A dose-effect determination revealed an overall decrease in responding for cocaine for the lesion group, but the integrity of the typical dose-effect relationship was preserved. The effect of ibotenic acid lesions on cocaine self-administration was also assessed using a progressive ratio procedure. In this test, the lesion group displayed a significant decrease in the highest FR value obtained.

The same experimental protocol was used to assess the effects of ibotenic acid lesions of the ventral pallidum on intravenous heroin (0.06 mg/kg/inj) self-administration. Preliminary results indicate that ventral pallidal lesions also produce a significant decrease in heroin self-administration when compared to vehicle control animals.

A decrease in response rate for drug self-administration following neurotoxic lesions is interpreted as an attenuation of the reinforcing properties of the self-administered compound. These results suggest that neuronal elements in the region of the nucleus accumbens that project to the ventral pallidum are critical for mediating the reinforcing properties of cocaine and heroin and that the nucleus accumbens-ventral pallidum circuit may be a common second-order link for both stimulant and opiate reward.

475.3 EVIDENCE FOR D2-RECEPTOR INVOLVEMENT IN TOLERANCE TO THE DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE. D. M. Wood and M.W. Emmett-Oglesby. Dept. of Pharm., Texas Col. of Osteopathic Med., Ft. Worth, TX 76107.

Rats were trained to discriminate the stimulus properties of cocaine, 10 mg/kg, using a food-rewarded 2-lever choice task. Responding under an FR10 schedule was reinforced on one lever following cocaine injection and was reinforced on the other lever following saline injection. After training, cocaine generalized to the training stimulus in a dose-dependent manner. The specific D2 receptor agonist, piribedil (20 mg/kg), produced full substitution for the cocaine stimulus; however, the specific D1 receptor agonist, SKF-38393 (40 mg/kg) produced only partial substitution for the cocaine stimulus (88% and 67% cocaine-lever responding, respectively). Higher doses were toxic and resulted in no lever selection during the 10 min. test session. When cocaine, 20 mg/kg/8-hr, was given for 7 days, both the cocaine and the piribedil dose-effect curves shifted approximately 2-fold to the right; in contrast, the dose-effect curve for SKF-38393 did not shift significantly. When piribedil, 20 mg/kg/8-hr, was given for 7 days, both the cocaine and the piribedil dose-effect curves shifted approximately 2-fold to the right. However, when SKF-38393, 40 mg/kg/8-hr, was given for 7 days, neither the cocaine nor the SKF-38393 dose-effect curves shifted significantly. In experiments with dopamine receptor antagonists, haloperidol (0.64 mg/kg) and sulpiride (20 mg/kg) partially blocked the cocaine cue when given acutely. Again, higher doses of these drugs were behaviorally disruptive. Simultaneous administration of haloperidol (0.64 mg/kg/day) or sulpiride (20 mg/kg/day) and cocaine (20 mg/kg/8-hr) for 7 days produced no significant shift in the cocaine dose-effect curve. When haloperidol, 0.64 mg/kg/day, or sulpiride, 20 mg/kg/day, was given alone for 7 days, the dose-effect curve for the detection of cocaine shifted approximately 2-fold to the left. These data suggest that tolerance and sensitization to the discriminative stimulus properties of cocaine are regulated through D2-receptor mechanisms.

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475.4 EVIDENCE FOR SITE SPECIFIC MEDIATION OF THE DISCRIMINATIVE STIMULUS PRODUCED BY COCAINE: THE ROLE OF THE NUCLEUS ACCUMBENS. M.W. Emmett-Oglesby and D.M. Wood. Dept. of Pharm., Texas Col. of Osteopathic Med., Ft. Worth, TX 76107.

Rats were trained to detect an intraperitoneal (i.p.) administration of cocaine, 10.0 mg/kg, using a two-lever choice discrimination procedure in which food reinforcement was only delivered following 10 responses on the correct lever (FR10): one lever was correct after cocaine injection, and the other lever was correct after saline injection. Following training, cocaine was generalized to the cocaine training stimulus in a dose-dependent manner. Subsequently, bilateral cannulae were implanted using the technique of Czech and Stein (Pharmacol. Biochem. Beh. 20: 811, 1984) and the coordinates from Paxinos and Watson (The Rat Brain in Stereotaxic Coordinates, 1982). Bilateral cannulae were placed in the prefrontal cortex (A = 2.0, L = 2.0, V = 3.0 mm), nucleus accumbens (A = 0.2, L = 3.0, V = 5.5 mm) and caudate-putamen (A = 0.2; L = 3, V = 4). Intracerebral microinjections of cocaine in the nucleus accumbens were also generalized to the i.p. training dose in a dose-dependent manner with maximum generalization occurring with 10 ug of cocaine per side (88% cocaine-lever responding); however, intracerebral injections of cocaine in the prefrontal cortex or caudate-putamen produced only partial cocaine-lever responding (50 and 38% cocaine-lever responding, respectively). These data suggest that the stimulus properties of cocaine administered centrally are generalized in rats trained by peripheral administration, and they support the hypothesis of central mediation of the cocaine stimulus. Furthermore, these data suggest that the mediation of the cocaine stimulus is site specific in dopaminergic brain sites, possibly specifically in nucleus accumbens. The partial substitution of cocaine in the prefrontal cortex and caudate-putamen may either reflect partial mediation of the cocaine stimulus in these brain areas or the diffusion of cocaine, a lipophilic substance, into the accumbens.

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475.5 SENSITIZATION TO THE BEHAVIORAL EFFECTS OF COCAINE IS ASSOCIATED WITH ALTERED DOPAMINE METABOLISM AND RELEASE IN RAT BRAIN. Kenneth M. Johnson and Lawrence D. Snell.* Dept. of Pharmacol. and Toxicol., Univ. of Texas Medical Branch, Galveston, Texas 77550.

The purpose of this study was to test the hypothesis that behavioral sensitization to cocaine is associated with altered dopamine (DA) metabolism in the striatum and nucleus accumbens. In two separate experiments we administered either water or cocaine (15 mg/kg) twice/day for ten days. On the morning of the eleventh day rats were given either cocaine (C) or water (W) and were either killed 15 min later (Exp 2) or rated behaviorally and killed 72 hr later (Exp 1). In either case, the brains were removed and the striatum, nucleus accumbens, medial prefrontal cortex, amygdala, and hippocampus frozen for HPLC-EC determination of DA, DOPAC, HVA, 5-HT, 5-HIAA, and NE. In Exp 2, part of the striatum was cut into 0.4 mm slices for measurement of DA release. Both experiments consisted of three groups: chronic water-acute water (W-W), chronic water-acute cocaine (C-C), and chronic cocaine-acute cocaine (C-C); N=5 or 6/group. Even though behavioral rating only was done in Exp 2, it was evident in both experiments that this regimen produced clear sensitization to cocaine. For example, 15 min post-administration, the group scores were 3.6 $\pm$ 0.3 (W-W), 3.8 $\pm$ 0.2 (W-C), and 7.5 $\pm$ 0.2 (C-C); $p<0.05$.

In Exp 2, in which the rats were killed 15 min after administration, dopamine metabolism (as assessed by the (DOPAC + HVA)/DA ratio) was decreased significantly in both the nucleus accumbens and striatum by cocaine in water pretreated rats (W-C) relative to control (W-W). However in cocaine pretreated rats (C-C), cocaine significantly inhibited DA metabolism in the accumbens but not the striatum. This may suggest an alteration in the striatal DA uptake mechanism. From Exp 1, it could be seen that the acute effect (15 min) of cocaine on striatal metabolism was no longer evident 72 hr later. However, the apparent metabolic state of the nigrostriatal pathway was at a new, lower (~30%) steady-state in the rats that received repetitive cocaine. In the accumbens, 72 hr following repetitive cocaine administration; DA, DOPAC, and HVA were all diminished by 39-46% (with no change in the (DOPAC + HVA)/DA ratio). This DA depletion was not evident 15 min following the 21st cocaine administration, suggesting that it takes a period of "withdrawal" before the change becomes evident.

Seventy-two hr following the last injection, amphetamine (1 M)-induced ^{3}H-DA release was 2.75 $\pm$ 0.28 (W-W), 2.89 $\pm$ 0.47 (W-C) and 6.27 $\pm$ 0.84 (C-C), $p<0.02$. This data, together with the metabolic data, suggests that repetitive cocaine administration may shift DA from a metabolically active pool to a pool more easily released by amphetamine. Alternatively, there may be changes in the DA carrier or other substrates of the amphetamine release process. (Partially supported by DA-02073).

475.6 COCAINE QUIETS THE ISOLATE'S CRIES AND PROMOTES NALTREXONE-REVERSIBLE POSITIVE ASSOCIATIONS IN RAT PUPS. P.Kehoe, L.Shaw* & S.Sakurai*. Dept.of Psychology, Trinity College, Hartford,Ct.06106.

Maternally isolated rat pups emit ultrasonic vocalizations. Opiates, at low doses, reduce these cries significantly. At these same low doses, opiates produce positive associative learning in the neonatal rat. In an effort to examine such neonatal psychobiological effects of the stimulant cocaine, the following experiments were done. In the first experiment Day 10 rats were individually isolated for 10 minutes in a warm environment (33° C). Pups were pretreated intraperitoneally with cocaine 2.5 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg, or saline 15 minutes prior to isolation. Ultrasonic vocalizations were monitored through a QMC Bat Detector and recorded. Activity levels were also observed and recorded. Cocaine at all doses significantly reduced the number of emitted cries during the entire 10 minutes of isolation. In fact, compared to the saline controls emitting 240 cries during the 10 minutes the lowest dose, 2.5mg/kg cocaine caused a reduction to 77 cries, while 5, 10, and 20 mg/kg administered pups were virtually quiet. Pups' activity scores were inversely correlated to their number of cries. That is, the quiet cocaine pups were significantly more active than the saline controls.

In a second experiment saline or cocaine in the above doses was administered to Day 5 rats at the onset of exposure to a novel odor which lasted for 30 minutes. Some of the pups were administered the opioid antagonist, naltrexone, prior to the cocaine or saline injection. After the odor exposure the pups were returned to their dam. Five days later they were tested for odor preference. A dose-response preference was seen, in that the lowest dose (2.5mg/kg) and highest dose (20 mg/kg) spent 30% and 28%, respectively, of the test time over the associated odor which did not differ from the controls (24%). The 5mg/kg and 10 mg/kg cocaine-treated pups highly preferred the associated odor choosing to spend 73% and 66%,respectively, in that part of the environment. These preferences were not seen in the naltrexone-cocaine treated pups. Thus, cocaine while producing hyperactivity in the isolated neonate abolishes ultrasounds and can support positive associative learning which is precluded by opioid antagonism.

475.7 6-HYDROXYDOPAMINE LESIONS OF THE NUCLEUS ACCUMBENS ALTERS THE EFFECTS OF COCAINE ON SCHEDULE-CONTROLLED BEHAVIOR. M. Matthews*, C.S. Sebastian, N.E. Goeders and S.I. Dworkin. Departments of Psychiatry and Pharmacology, Louisiana State University School of Medicine, Shreveport, LA 71130.

Drug self-administration procedures have often been used to investigate central mechanisms of reinforcement. Both specific neurotoxin lesions of discrete brain regions and centrally acting receptor antagonists can alter the rate and pattern of drug intake. Decreases in responding observed after these manipulations are sometimes suggested to demonstrate a decrease in reinforcing efficacy which implies that the neuronal systems affected by the manipulation are involved in central mechanisms of reinforcement. However, most drugs have multiple effects on behavior so that specific neurobiological manipulations may also alter some of the unconditioned behavioral effects of the drug. This study determined the effects of cocaine on schedule-controlled behavior before and after 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens.

Male F-344 rats were trained on a multiple fixed-interval 4 min, fixed-ratio 15 schedule of food presentation. The prelesion dose-response curves for cocaine (3.0-56.0 mg/kg) indicated that cocaine increased responding maintained by the interval contingency at doses that had no effect on or decreased responding maintained by the ratio contingency. While vehicle lesions did not alter the effects of cocaine, bilateral 6-OHDA lesions of the nucleus accumbens enhanced the rate increasing effects of cocaine on fixed-interval responding and attenuated the rate decreasing effects of the drug on responding maintained by the ratio contingency.

6-OHDA lesions of the nucleus accumbens can alter the unconditioned behavioral effects of cocaine. Therefore, changes in the rate-dependent effects of the drug may be partially responsible for the effects of similar lesions on drug self-administration. Supported by USPHS Grant DA 03631.

475.8 THE EFFECTS OF 6-HYDROXYDOPAMINE LESIONS OF THE NUCLEUS ACCUMBENS ON THE DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE USING A CUMULATIVE DOSING PROCEDURE. C. Bimle*, N.E. Goeders and S.I. Dworkin (SPON: G. Trapp). Louisiana State University School of Mendicine, Shreveport, LA 71130.

Neurotoxin lesion methodologies have been used to assess the role of discrete brain sites and neurotransmitter systems in the modulation of drug reinforcement mechanisms using the drug self-administration procedure. Unfortunately, the standard self-administration procedure may confound several of the behavioral effects that drugs exert. A decrease in drug intake following a lesion may not be indicative of changes in reinforcement efficacy but may possibly indicate a change in the discriminative stimulus or rate influencing properties of the drug. This study examined the effects of 6-hydroxydopamine (6-OHDA) lesions of the nucleus accumbens on the stimulus properties of cocaine.

Adult male F-344 rats were trained to discriminate cocaine (10 mg/kg, IP) from saline using a two-lever, fixed-ratio, drug discrimination paradigm. Single or multiple daily training sessions were conducted prior to which the rats received an injection of either saline or drug. Once the cocaine discrimination was acquired, generalization curves were determined using a cumulative-dosing procedure. The rats were injected with saline at the start of these determinations. Following three minutes of exposure to the fixed-ratio schedule, cocaine (.82 mg/kg) was delivered during 1 min time-out periods. Additional cocaine infusions were delivered during successive time-out periods until the rats chose the cocaine lever during two successive trials. The rats were given 6-OHDA lesions of the nucleus accumbens and the generalization gradients were redetermined. The lesion produced significant decrements in the discriminative stimulus properties of the drug indicating that dopaminergic innervations of the nucleus accumbens are involved in the stimulus properties of cocaine. (Supported by USPHS grant DA 03631).

475.9 CHLORDIAZEPOXIDE MODIFIES THE INTRAVENOUS SELF-ADMINISTRATION OF COCAINE IN RATS. K. H. McAllister*, S. M. Dworkin*, S. I. Dworkin and N. E. Goeders, (SPON: L. J. Embree) Departments of Pharmacology and Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130

Cocaine is a potent reinforcer that maintains stable patterns of self-administration over long periods of time. Most investigations of the neuronal substrates mediating the behavioral properties of the drug have focused on catecholaminergic pathways. However, neuropharmacological and receptor binding studies also indicate a complex interaction between cocaine and benzodiazepines. For instance, the co-injection of cocaine and chlordiazepoxide (CDP) increases locomotor activity above the actions of each drug alone (D'Mello and Stolerman, Br. J. Pharmac., 54:131, 1977). Furthermore, the chronic administration of cocaine alters benzodiazepine receptor density in specific brain regions (Goeders et al, Soc. Neurosci. Abstr., 1987) and these effects may be mediated by dopaminergic mechanisms since depletion of brain dopamine by 6-hydroxydopamine reduces benzodiazepine receptor densities (Sabato et al, Eur. J. Pharmacol., 73:381, 1981). Therefore, it was of interest to investigate whether intravenous cocaine self-administration could be modified by benzodiazepine pretreatment.

Male rats, originally derived from the Fischer 344 strain, were implanted with chronic jugular catheters. The rats were allowed to self-administer cocaine (0.17 mg/0.2 ml infusion) on an FR4:LH 300 sec schedule during 2.5 hr sessions. Completion of the response requirement resulted in a 5 sec infusion of cocaine accompanied by illumination of a house light and onset of a 20 sec tone. When stable patterns of self-administration were obtained, the rats were injected with CDP (0.1-30.0 mg/kg, i.p., 15 min prior to testing) dissolved in physiological saline (which served as the vehicle control).

Low doses of CDP (0.3-1.0 mg/kg) significantly increased the frequency of cocaine infusions/session, while higher doses (5.6-10.0 mg/kg) decreased the number of infusions. Increasing the cocaine dose (0.34 mg/infusion) attenuated the CDP-induced reductions in self-administration. The reductions in self-administration at both doses of cocaine were unaccompanied by increases in the number of limited holds indicating that the effects of the CDP were not likely due to decreases in response rate. These effects of CDP on cocaine self-administration may therefore involve a modification of the stimulus properties or reinforcing efficacy of cocaine. These data provide further support for the involvement of benzodiazepine binding sites in the behavioral effects of cocaine.

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475.10 CONDITIONED PLACE PREFERENCE FOR COCAINE IN RATS: OPEN TEST WITH KNOWN EXPECTED BASELINE CHOICE VALUES. R.C. Michaelis, F.A. Holloway, R.D. Harland*, and J.R. Criado. (SPON: J.A. Holloway) Univ. Oklahoma Health Sciences Center, Oklahoma City, OK 73190

One of the major problems associated with the conditioned place preference (CPP) paradigm in assessing the reward properties of drugs has been marked individual differences in baseline preference for stimuli paired with drug or non-drug conditions. Often, baseline choice behavior is assumed to be random. The present study used a new CPP pradigm (P. Venzina & J. Steward, Neurosci. Abst. 12:1278, 1986), employing a unique strategy in order to obtain a known expected test choice value for each animal.

The four CPP chambers used in this study were 2' x 2' square with four quadrant floors (rods or wire mesh) which could be arranged in any order. The quadrant floor sections were mounted on a frame attached to contact relays and those signals were fed into a Commodore-64 computer which recorded all latencies and activity. Male, albino rats were randomly assigned to three groups (N=8/group). On training days, all four quadrants were either rod or mesh. One group received cocaine (COC)/rod and saline (SAL)/mesh pairings (COC+ROD), another COC/mesh and SAL/rod (COC+MESH), and the final group was designated as CONTROL. The 10 mg/kg COC or SAL was injected IP 2 min prior to the 20-min training or TEST sessions. The experiment lasted 3 weeks which permitted 3 test days. Each week, 2 COC and 2 SAL training sessions preceded the no injection TEST day. The COC+rod and COC+mesh groups received conditioning trials each week. On Week 1, one-half the CONTROL rats received COC+rod pairing and the others, COC+mesh pairings; on Week 2, these pairing conditions were reversed; and on Week 3, all CONTROL rats had COC and SAL paired with both rod and mesh cues. Each week, on the second COC session, time spent in the least preferred quadrant was designated as the baseline expected value for each rat, and that unique quadrant (rod or mesh) was used as the unique quadrant on the test day. The results indicate that on Week 1 all three groups spent significantly ($p < .05$) longer times in the unique CS+ quadrant than on baseline. This trend continued for the COC+ROD and COC+MESH groups on Weeks 2 and 3 ($p < .05$). However, the CONTROL group displayed a reduced preference for the CS+ quadrant on Week 2 and on Week 3 spent virtually identical periods of time in the unique quadrant on baseline and test sessions.

Besides demonstrating that stimuli paired with cocaine can become "secondary reinforcers," these results provide a refinement of the CPP paradigm by utilizing a known choice value for baseline against which conditioned preferences can be assessed. By selecting the least preferred quadrant during drug sessions as the baseline reference, CPP can be assessed in a conservative fashion against each animal's baseline behavior.

475.11 SENSITIZATION TO COCAINE AND APOMORPHINE: NO INTERACTION IN LESIONED RATS. P.B. Silverman. Psychopharmacology, University of Texas Mental Sciences Institute, Houston, TX 77030.

Repeated administration of stimulants often results in a progressively augmented behavioral response. The issue of whether or not such augmentation is similarly mediated for stimulants that have different mechanisms of action is not established. It has been suggested that cocaine sensitization is a consequence of the development of a denervation type supersensitivity of postsynaptic dopamine (DA) receptors. Apomorphine (APO) sensitization, on the other hand, has been proposed to result from desensitization of normally inhibitory presynaptic DA receptors.

A limited test of these suggestions was conducted using the 6-hydroxydopamine (OHDA) lesioned rotating rat model. One might expect that rats sensitized to cocaine would show a decreased response to APO relative to their pre-sensitization response, since sensitization of the intact side should make the sides more equally sensitive to APO. Repeated APO administration might be expected to result in a decrease in rotational response to APO, because development of presynaptic hyposensitivity, occurring only on the intact side, should make both sides more equally responsive to APO. At the same time, repeated APO should increase cocaine sensitivity because of development of presynaptic hyposensitivity on only the intact side.

Male S-D rats, under pentobarbital anesthesia, were lesioned in one substantia nigra by infusion of 6-OHDA. One group of animals was tested with APO before, and again after, 2 weeks of daily cocaine (10 mg/kg) administration. These animals showed a highly significant increase in rotational response to cocaine, and no change in response to APO. A second group of animals was tested with cocaine before, and again after, two weeks of daily APO (0.1 mg/kg) administration. The rotational response to APO increased significantly, but these APO-sensitized animals showed no change in response to cocaine. Sensitization to these two compounds thus appears to develop via independent means.

475.12 DIFFERENTIAL EFFECTS OF COCAINE ON INHIBITION OF SYNAPTOSOMAL ^{3}H-AMINE ACCUMULATION AND DIFFERENTIAL COCAINE PREFERENCE IN FOUR INBRED MOUSE STRAINS. T. Z. Bosy*, W. R. Wilson* and J. A. Ruth. The School Of Pharmacy, University of Colorado, Boulder, CO 80309-0297.

We have previously shown that cocaine differentially affects locomotor activity in BALB, C57BL, C3H and DBA strains of mouse. These differences were not due to differences in metabolism as measured by brain concentrations of ^{3}H-cocaine at the time of maximal locomotor response. To assess possible biochemical correlates in the differential locomotor response of these mice to cocaine, the effect of cocaine on the inhibition of synaptosomal accumulation of ^{3}H-(-)-norepinephrine (NE), ^{3}H-dopamine (DA) and ^{3}H-5-hydroxytryptamine (5HT) was studied in each of the four inbred strains. A significant strain difference was observed in the efficacy of cocaine-induced inhibition of synaptosomal ^{3}H-NE accumulation, with maximal inhibition of uptake ranging from 40% (BALB) to 75% (C57BL) of control values. In each case the apparent IC_{50} value was apx. 5×10^{-7}M. Strain differences in both efficacy and potency were observed in the cocaine inhibition of synaptosomal ^{3}H-DA uptake. C57BL mice were most sensitive (80% max. inhibition of uptake, IC_{50} 10^{-7}M), while C3H mice were least sensitive (65% max. inhibition, IC_{50} 5×10^{-7}M). The greatest strain differences were observed with cocaine inhibition of ^{3}H-5HT accumulation. DBA mice were extremely sensitive (80% max. inhibition, IC_{50} 5×10^{-8}M), while C57BL were much less sensitive (60% max. inhibition, IC_{50} 10^{-6}M). In a two-bottle preference paradigm, C57BL demonstrated impressive cocaine preference at concentrations of 50, 100, 200 and 400 mg/l versus water (preference ratios 1.5 - 8.7). C3H mice demonstrated a less pronounced preference over the range of 50 - 200 mg/l (preference ratios 1.8 - 2.9), and avoided cocaine at 400 mg/l. DBA and BALB mice avoided cocaine-containing solutions (preference ratios 1). The results support a role for genetic influences in both the biochemical and behavioral responses to cocaine in these strains of mice.

475.13 EFFECT OF SEVERAL CNS AGENTS ON P300 IN RATS. F. Wirtz-Brugger*, M. Cornfeldt*, K. McCormack*, S. Szewczak* and S. Fielding. Dept. of Biological Research, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

The P300 (P3) is a positive, long-lasting component of the averaged evoked response that occurs, on the average, at 300 msec in response to a rare, unpredictable stimulus that is relevant to an ongoing task. The P3 has been associated with cognitive processes, including memory, recall, information processing and attention. Changes in both latency and amplitude of the P3 have been correlated with diseases that effect cognition such as dementia, delerium and psychosis. Previously, we presented a model for generating P3 in rats and the effects on memory-enhancing drugs in this procedure. We now report on an extension of this methodology and additional drugs implicated in cognitive activity.

We used essentially the same procedure previously reported but included additional electrode sites; they were: Pz, Cz, Fz and P3, P4, F3 and F4. The "odd-ball" tone-tone paradigm was used to generate the P3 (1 KHz tone freq. -3 KHz tone rare). Drugs tested were tacrine (5 mg/kg ip), naloxone (5 mg/kg ip), cocaine (5 mg/kg sc), forskolin (0.1 mg/kg ip) and saline (0.1%) as a control. Statistical comparisons of the areas under the P3 curve were made using a paired t-test. In addition, several subjects from each group were analyzed using brain-mapping technology (BEAM). There were no significant changes with saline. Tacrine, forskolin and cocaine significantly ($P < 0.05$) increased the area of the P3 recorded from the central channels. Significant ($P < 0.05$) enhancement of the P3 recorded from the lateral channels occurred with tacrine (F3, F4), cocaine (F3, F4, P4) and forskolin (P3, P4, F3).

Brain maps of individual subjects clearly demonstrated that tacrine and cocaine had the greatest effect on positivity in the central and frontal areas while forskolin's effects were more evenly distributed over the entire brain. Naloxone significantly ($P < 0.05$) decreased the area of the P3 in Pz and ($P < 0.1$) Cz. It had no significant effects on any other channels.

These data suggest that compounds with different pharmacological profiles but which have been reported to have positive effects on CNS function cause significant increases in CNS electrical activity which is related to cognitive activity. Differences in patterns of the increases may reflect the fundamentally different nature of the profile of each compound.

BEHAVIORAL PHARMACOLOGY: ADDICTION

476.1 A COMPARISON OF THE DISCRIMINATIVE STIMULI OF (+) AND (-) 3,4-METHYLENEDIOXYAMPHETAMINE (MDA) WITH THOSE OF HALLUCINOGENIC AND STIMULANT DRUGS. J. Broadbent, E. K. Michael*, J. H. Ricker*, J.B. Appel Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, SC 29208

Due to the reported abuse of the amphetamine derivative 3,4-(MDA), the behavioral actions of this compound have been of interest to this laboratory (see abstract by Callahan and Appel). Several reports from other laboratories have indicated that the discriminative stimuli induced by racemic (±) MDA mimic those of (+) amphetamine, LSD and cocaine. Further, the 'stimulant-like' component of the (±) MDA cue appears to due to its (+) isomer while the 'hallucinogenic' component is caused by the (-) isomer (Glennon and Young, 1984). To further test this hypothesis, the two isomers of MDA (+ and -) were given to rats (n=8) trained to discriminate amphetamine (1 mg/kg), cocaine (10 mg/kg) or LSD (0.16 mg/kg) in a water-reinforced (FR 20), lever-pressing task. Only animals reaching the criteria of 90% correct responding during the completion of the first FR on 10 consecutive days were tested (during extinction sessions). Both (+) and (-) MDA failed to mimic the amphetamine cue at the doses of MDA tested (0.5,1.0,1.5 and 2.0mg/kg). However, animals trained to discriminate cocaine from saline did choose the drug-appropriate lever following both the (+), and to a lesser extent, the (-) isomer of MDA. In addition both isomers of MDA engendered drug-appropriate responding in animals trained to discriminate LSD from saline.

These data suggest that both isomers of MDA bear at least some similarity to LSD and cocaine. Contrary to previous reports, however, very little similarity of either (+) or (-) MDA to d-amphetamine was observed at the doses tested.

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476.2 DIFFERENCES IN THE STIMULUS PROPERTIES OF 3,4-METHYLENE DIOXYAMPHETAMINE (MDA) AND N-METHYL-1-(3,4-METHYLENEDIOXY AMPHETAMINE) MDMA IN ANIMALS TRAINED TO DISCRIMINATE HALLUCINOGENS FROM SALINE. P. M. CALLAHAN and J. B. APPEL. Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, SC 29208.

Purportedly, the behavioral effects of (±) MDA (the 'love' drug) has both stimulant effects that are due primarily to its (+) isomer and hallucinogenic effects that are attributed to its (-) isomer. MDMA ('ecstasy'), a structural analog of MDA, appears to have no hallucinogenic properties. In this paper, evidence is presented that questions these conclusions. Two groups of rats were trained to discriminate either LSD (0.08 mg/kg, i.p.) or mescaline (10.0 mg/kg, i.p.) from saline in a two-choice, water-reinforced (FR 20) drug discrimination task. When animals in the LSD group (N=8), were given substitution (generalization) tests with (-) MDA (0.5-1.75 mg/kg), responding occurred primarily on the drug-appropriate lever; tests with (+) MDA (0.5-1.75 mg/kg) produced predominantly saline-lever responding. Both isomers of MDMA (0.5-2.0 mg/kg) also engendered responding on the saline lever. In the mescaline group (N=8), (-) MDA (0.5-2.0 mg/kg), (+) MDA (0.5-2.0 mg/kg), (-) MDMA (1.0-2.0 mg/kg) and (+) MDMA (0.25-1.0 mg/kg) mimicked the training drug. Given these results, and the fact that (+) MDA and (±) MDMA have been reported to have stimulant properties, animals in the mescaline group were tested with 0.5-2 mg/kg of amphetamine. No dose of this compound produced drug-like responding.

These results indicate that (-) MDA appears to have effects that are similar to both LSD and mescaline and thus confirm previous findings that (-) MDA is hallucinogenic. Results with the other isomers are less easily reconciled with the existing literature. While the discriminable effects of (+) MDA may not resemble those of LSD (at this training dose), they are similar to those of mescaline; therefore, it cannot be concluded that the hallucinogenic potency of MDA is caused solely by the (-) isomer. Since both isomers of MDMA mimic mescaline, this compound does not necessarily lack hallucinogenic effects. It is unlikely that the substitution of MDA and MDMA for mescaline is the result of structural similarities among these compounds since the closely-related compound amphetamine does not have mescaline-like effects.

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476.3 EFFECTS OF MESCALINE ON VISUALLY EVOKED POTENTIALS IN THE AWAKE CAT: AN ANALYSIS OF PRINCIPAL COMPONENTS. D.M. Wilkison. Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226

In earlier studies we have reported that drugs of abuse differentially alter sensory activity in cortical and thalamic structures in chloralose-anesthetized cats. Investigations of the actions of mescaline in chronically prepared, awake cats have been undertaken to extend these observations and evaluate correlates of behavioral actions.

Cats, adapted to sling restraint, were presented visual (flash), auditory (click) and somatic (electrical stimulation) sensory stimulation on a randomized schedule with probabilities of 20%, 60% and 20%, respectively. Principal component analysis (SAS Institute, Inc.) of visually evoked potentials from six cortical and thalamic sites collected before and after 16 mg/kg mescaline was performed for each cat (n=4) separately. Six factors found common to all experiments were compared. Three factors which were weighted heavily on long-latency, 150-400 msec, components were increased in the visual cortex and medial thalamus while decreased at the anterior suprasylvian cortex. A midlatency component, 40-90 msec, was increased in the suprasylvian cortex. Short-latency factors had variable responses to mescaline.

The data show that mescaline most consistently produced an increase in long-latency responses in the visually evoked potential recorded from cortical and thalamic sites. These potentials appear as phasic or oscillatory waves (5-6 cps) most prominently in intralaminar thalamus. Nucleus reticularis of the thalamus also displayed oscillatory responses to flash after mescaline. Mescaline actions appear to involve a globally projecting system producing slow wave oscillatory cycles in response to flash stimulus. Auditory and sometic stimulation do not produce such activity. This study was supported by NIDA ROl DA 03785.

476.4 **ANTAGONISM OF LSD-INDUCED BEHAVIORAL CHANGES IN PRIMATE SOCIAL COLONIES BY CHRONIC TRAZODONE TREATMENT.** **R.F. Schlemmer, Jr., C. Kane*, J.E. Young*, N.L. Katz, and J.M. Davis. Dept. of Pharmacodynamics, Univ. of Ill. at Chicago and Ill. State Psychiatric Inst., Chicago, IL. 60612.**

Electrophysiological studies have demonstrated that chronic trazodone (TRAZ) administration antagonized the action of LSD on dorsal raphe neurons in the rat (Dowdall and deMontigny, Neurosci. Abst. 10:17, 1984; Blier et al., J. Clin. Psychopharmacol., in press). The present study examined the effect of acute and chronic administration of TRAZ on LSD-induced behavior in monkeys. Two stable social colonies of 4 adult Stumptail macaques each served as subjects for the study. The experiment was divided into 3 parts. Following observation of undrugged behavior (BASE), each monkey received (i) acute administration of d-LSD, 0.01 mg/kg, (ii) acute administration of TRAZ, 2 or 4 mg/kg, followed by LSD and (iii) administration of TRAZ, 2 or 4 mg/kg, twice daily for 15-16 days with LSD being given on the last day of treatment. TRAZ was given 3 hrs. and LSD 15 min. prior to observation. All drugs were given i.m. and only 2 monkeys received treatment on a given day. A 60 min. behavioral observation session was conducted on designated days by a "blind" observer using a checklist of 40 behaviors. LSD induced similar behavioral changes as previously reported for this species (Schlemmer and Davis, Pharmacol. Biochem. Behav., 24:381, 1986). Not all monkeys responded similarly to LSD after chronic TRAZ. Treated monkeys could be divided into 3 groups based on appearance and observation data. Three responders appeared essentially unaffected by LSD following TRAZ and remained integrated in colony behavior, 2 monkeys only had a partial response to LSD, while the 3 remaining monkeys were nonresponders and had a full LSD response despite TRAZ treatment. In responders, chronic TRAZ completely antagonized the hallucinogen-specific behavior limb jerks, but more importantly restored initiated social grooming which had been eliminated by LSD and returned distancing scores which were increased by LSD to BASE levels. LSD-induced reductions in self grooming were antagonized by both acute and chronic TRAZ. Interestingly, LSD-induced body shakes were not antagonized by acute or chronic TRAZ in any monkeys. The results of this study may have important implications for both hallucinogenic and antidepressant drugs. These findings suggest that TRAZ may have utility in the treatment of LSD intoxication. Although differences between responding and non-responding monkeys remain unresolved at this time, they may be useful in elucidating factors which are responsible for similar results seen with clinical antidepressant drug treatment.

476.5 ANALYSIS OF THE FUNCTIONAL OUTPUT FROM THE SUBSTANTIA NIGRA FOLLOWING ACTIVATION BY PHENCYCLIDINE. H.D. Everist and A. Pert. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

We have previously shown that direct unilateral injections of phencyclidine into the substantia nigra of rats produce profound rotational behavior contralateral to the injection. Such rotational behavior may be mediated through several different pathways originating from either the zona compacta or zona reticulata. In order to define these functional outputs which are activated by PCP, rats were implanted with unilateral cannulae guides aimed for the SN as well as with i.v. jugular catheters. Following recovery, the animals were injected in the SN with 25 n moles of PCP. Three minutes later the same rats were also injected i.v. with 100 uCi/kg of [^{14}C]-2-deoxyglucose (2-DG). Forty five minutes following administration of 2-DG the animals were sacrificed. The brains were prepared for autoradiographic analyses using standard procedures. Injections of PCP into the SN produced rotational behavior in rats which was accompanied by alterations in the metabolic activity of several brain structures. Substantial increases in metabolic activity were found in the caudate nucleus ipsilateral to the injection. Somewhat smaller increases in activity were also seen in the ipsilateral globus pallidus and n. accumbens. No consistent changes could be detected in thalamic nuclei. The SN caudal to the injection site appeared to be profoundly activated. Significant activation could also be detected in the ipsilateral pontine reticular nuclei as well as the medial and lateral olivary complex. While intranigral PCP appeared to activate nigrostriatal pathways, it is unlikely that these participate in the rotational response since 6-OHDA lesions of the medial forebrain bundle or hemi-transections rostral to the SN did not alter rotational output eliciated by intranigral PCP. It appears more likely that projections to pontine reticular structures are critical since hemi-transections caudal to the injection were effective in attenuating the effects of intranigral PCP.

476.6 THE EFFECTS OF SELF-ADMINISTERED PHENCYCLIDINE (PCP) ANALOGUES ON CORTICAL EEG OF THE RAT. K.L. Marquis, R.P. Gussio, M.G. Webb and J.E. Moreton. Dept. of Pharmacol. & Toxicol., Univ. of Maryland Sch. of Pharmacy, 20 N. Pine St., Baltimore, MD 21201.

The self-administration of PCP is associated with changes in cortical EEG (Marquis et al., Fed. Proc. 46:399, 1987). The present study investigated the changes in cortical EEG produced by the self-administration of the PCP analogues ketamine and 1-(1-phenylcyclohexyl) morpholine (PCM) in the rat.

Four female Sprague-Dawley rats implanted with chronic intravenous cannulae and cerebrocortical electrodes were trained to lever press for cocaine (1.0 mg/kg/inj) on an FR10 schedule of reinforcement. Ketamine (3.0 mg/kg/inj) then replaced cocaine in daily 3 hr sessions. Periodically, PCP (0.5 mg/kg/inj), ketamine (4.0) or PCM (4.0) was made available for self-administration during a single session while the cortical EEG was time-coded and recorded on a Grass model 7D Polygraph and Vetter Model D FM recorder. Lever presses and injections were recorded on the polygraph record beneath the EEG tracings in order to locate sections of EEG signal for later analysis. Using a Nicolet Pathfinder I computer, the minute preceding and following each injection of a test session was digitized. Spectral quantities for frequency, power, mobility and complexity were determined in the 0 to 20 Hz frequency range. Also, relative power in the 0-5 and 5-10 Hz frequency bands was determined.

The self-administration of all three drugs resulted in increases in the total power expressed as a percentage of a pre-drug baseline. This increase in power was associated with a slowing of the predominant frequency to the low frequency range (0-5 Hz). Prior to the next injection total power declined but remained elevated above baseline. The results for power and frequency are summarized in the following table:

		TOTAL PWR (%)		PEAK Hz (average)	
DRUG	Inj/Hr (mean±SD)	PRE-	POST-	PRE-	POST-
PCP	3.7 ± 1.4	110-210	150-300	8	2
KET	5.4 ± 0.5	75-240	110-500	8	2
PCM	8.7 ± 2.7	210-860	300-900	2	2

Discriminant analysis of the spectral quantities grouping pre- and post-injection samples revealed a significant ($p < 0.01$) difference between pre- and post-injection cortical EEG characteristics for each subject self-administering ketamine or PCM and for 3 subjects self-administering PCP. Analysis of lower unit doses of each compound is in progress. These data indicate that rats will self-administer ketamine and PCM at a rate such that similar effects on cortical EEG are observed when compared to self-administered PCP. Supported by NIDA Grant DA 03173.

476.7 **EFFECTS OF PHENCYCLIDINE ON PUNISHED AND UNPUNISHED RESPONDING IN RATS.** J.H. Porter, J.L. Wiley* and R.L. Balster* (SPON:B.F. Kilpatrick). Depts. of Psychology and Pharmacology, Virginia Commonwealth Univ., Richmond, VA 23284.

Antianxiety drugs such as chlordiazepoxide (CDP) increase punished responding in animals. This antipunishment effect is also commonly seen with CNS depressants (e.g., barbiturates, ethanol). Phencyclidine (PCP) shares many properties with CNS depressants (Balster & Wessinger, 1983), and it has been shown that PCP increases rates of punished responding in pigeons (Chait & McMillan, 1979). However, this antipunishment or anticonflict effect has not been shown in rodents. The present study tested rats in a Geller-Seifter (1960) conflict test to determine if PCP would produce an increase in punished responding in rodents.

Twenty male rats (80% BW) were trained to respond under a multiple fixed-interval 60-sec (food only) fixed ratio 1 (food + shock) schedule of reinforcement. The components lasted 7 min and 3 min, respectively, with a total session length of 30 min. Initially, the rats were tested with four doses of CDP (0, 2.5, 5.0 and 10.0 mg/kg) administered intraperitoneally (IP) 30-min presession. Then, four doses of PCP (0, 1.0, 2.0 and 4.0 mg/kg) were administered IP 15-min presession. Response rates (responses/minute) on test days were analyzed with ANOVAs and Duncan's post hoc tests.

As shown in the table below, both PCP and CDP produced significant increases in PUNISHED (FR) responding. For UNPUNISHED (FI) responding, the high dose of PCP produced a significant decrease; the 2.5 mg/kg dose of CDP produced a significant increase in responding. These results demonstrate that PCP produces an antipunishment effect in rodents similar to that seen in pigeons. It is possible that the abuse of PCP may be in part related to its antianxiety effects.

PUNISHED (FR) AND UNPUNISHED (FI) RESPONDING (RESPONSES/MINUTE)

PCP	0	1.0	2.0	4.0 mg/kg
PUNISHED RESP	0.49	1.32	3.41*	1.56
UNPUNISHED RESP	17.5	20.6	17.4	8.95*
CDP	0	2.5	5.0	10.0 mg/kg
PUNISHED RESP	0.72	2.81*	4.72*	5.99*
UNPUNISHED RESP	19.1	25.7*	24.5	16.71

* Sign. Diff. from Vehicle ($p < .05$)

(Research supported by NIDA grant DA-01442)

476.8 THE EFFECTS OF THE ACUTE AND CHRONIC ADMINISTRATION OF PHENCYCLIDINE HYDROCHLORIDE (PCP) ON BODY TEMPERATURE IN THE RAT. R.N. Pechnick, R. George* and R.E. Poland*. Department of Pharmacology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024 and Division of Biological Psychiatry, Harbor-UCLA Medical Center, Torrance, CA

Phencyclidine (PCP) is a major drug of abuse in the United States. Many drugs of abuse affect thermoregulation and hyperthermia is frequently observed as a toxic manifestation of PCP use in humans. The purpose of the present study was to ascertain the effects of the acute and chronic administration of PCP in the rat. Saline or the same dose of PCP (1.0, 5.0, 10.0 or 20.0 mg/kg s.c.) was administered daily to male rats for 14 days. The temperature response following injection was measured on Days 1, 7 and 14. On Day 1 PCP administration produced dose-dependent hypothermia in the rats receiving 10.0 and 20.0 mg/kg s.c.; no hyperthermia was observed in any of the groups. In contrast, on Days 7 and 14 PCP produced dose-dependent hyperthermia in all groups. These results indicate that, similar to the pattern observed with opiates, with repeated administration the temperature responses to PCP shifts from hypothermia to hyperthermia (Supported by NIDA grant DA-04113).

476.9 A COMPARISON OF THE EFFECTS OF INTRA-ACCUMBENS INJECTIONS OF AMPHETAMINE AND MORPHINE ON REINSTATEMENT OF HEROIN INTRAVENOUS SELF-ADMINISTRATION BEHAVIOR. Jane Stewart, Center for Studies in Behavioral Neurobiology, Psychology Department, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

Studies from this laboratory have demonstrated that in rats previously trained to self-administer heroin intravenously, self-administration behavior can be reinstated by the application of morphine to the ventral tegmental area (VTA) (Stewart, Pharm. Biochem. & Behav., 1984). This finding suggested that activation of mesolimbic dopamine neurons might underlie the priming effects of i.v. injections of opiates and stimulants on such behavior (see de Wit & Stewart, Psychopharm. 1981, 1983).

In the present experiments, rats were trained to self-administer heroin via intravenous catheters and were then given sessions of extinction training during which they received i.v. injections of the saline vehicle. Following extinction training sessions, and after a period of at least 30 min. of no responding, bilateral microinjections of either 0.5 µl saline or 10 µg/0.5 µl amphetamine sulphate were made through cannulae aimed at the N. accumbens and angled to avoid the ventricles. Amphetamine, but not saline, was found to reinstate self-administration behavior for a period of about one hour. In contrast, bilateral intra-accumbens injections of either 5 or 10 µg/0.5 µl morphine sulphate to these same animals were found either to have no effect on responding or to lead to only infrequent responding that generally occurred after one hour into the session. These differences occurred despite the fact that both drugs increased locomotor activity measured in independent tests. It is important to note, therefore, that unlike the activity produced by VTA morphine and accumbens amphetamine, the activity produced by accumbens morphine occurs independent of the mesolimbic DA system (Kalivas et al, J. Pharm. Exp. Ther., 1983). Furthermore, unlike the effects of morphine applied repeatedly to the VTA, morphine applied repeatedly to N.accumbens does not lead to sensitization of the locomotor activity effect (Vezina et al, Brain Res. in press). The present results provide, therefore, not only further support for the idea that the reinstatement effects of opiates and stimulants on self-administration behavior are mediated by the mesolimbic DA system, but, in addition, might suggest that reinstatement by drug priming may be related to the ability of opiates and stimulant drugs to cause sensitization within this system.

476.10 CONDITIONED STIMULUS CONTROL OF MORPHINE HYPERTHERMIA. K. S. Schwarz and C. L. Cunningham. Dept. of Medical Psychology, Oregon Health Sciences Univ., Portland, OR 97201.

Classical conditioning has been shown to play a role in how an organism responds to drugs administered chronically. For example, after repeated exposures to drug in the presence of certain cues (e.g., a "distinctive" room), an animal will show tolerance or sensitization to the drug effects only in the presence of those cues. Placebo administration in the presence of drug-paired cues often results in a conditioned response which either mimics or is opposite the drug effect. The purpose of the present study was to determine whether drug responses could be conditioned when intravenous morphine administration was paired with an explicit conditioned stimulus (CS).

Adult male rats were implanted with a jugular vein cannula and a biotelemetry device (MiniMitter) for monitoring body temperature. The animals were housed 24 hrs/day in light- and sound-attenuating chambers in which all testing occurred. The CS was a compound light/white noise which lasted 15 min. The dose of morphine was 5 mg/kg. The rats were randomly assigned to one of two groups. The Paired group (n=7) received morphine 30 sec after the onset of the CS, i.e., morphine was administered and the CS remained on for an additional 14.5 min. The Unpaired group (n=8) received explicitly unpaired presentations of the CS and morphine, i.e., morphine was infused 75 min after CS offset. Every seventh day, the Paired group received a delayed-morphine test in which morphine was administered at the end of the 15-min CS to test for a conditioned response. At the end of the training phase, both groups were given morphine with and without the CS.

In general, morphine produced hyperthermia in both groups and the latency to reach maximum hyperthermia decreased during training. On the delayed-morphine tests, the Paired group showed a conditioned hyperthermic response to the CS, whereas the Unpaired group showed no change in temperature in response to the CS.

The results of the post-training tests revealed greater hyperthermia in the Paired group relative to the Unpaired group during the CS. In the absence of the CS, there was no difference between the groups in their response to morphine. These results are consistent with the suggestion that a conditioned response contributes to sensitization of morphine hyperthermia.

Overall, these results clearly show that learning is capable of affecting the response to morphine administered repeatedly. In contrast to previous studies, conditioned hyperthermia was elicited within 15 min by a discrete CS in a situation where the response was not confounded by handling or the stress of injection and where there was an appropriate control for nonassociative factors.

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476.11 THE ROLE OF OPIOID DELTA RECEPTORS IN THE PRODUCTION OF LOCOMOTOR HYPERACTIVITY OF THE C57BL/6J MOUSE. G. A. Mickley and M. A. Mulvihill, Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

Morphine induces a dose-dependant stereotypic locomotor hyperactivity in the C57BL/6J mouse (Teitelbaum et al., J. Comp. Physiol. Psychol.93:745-751, 1979). Although morphine is recognized as an opioid mu receptor agonist, it also binds at other subtypes of the opioid receptor. Here we used a relatively specific delta receptor agonist and antagonist as well as a sophisticated activity measuring apparatus to further investigate the neurochemical mechanisms of opiate-induced locomotion in the C57BL/6J mouse.

Male C57BL/6J mice (23 to 32g) were anesthetized and implanted with chronic bilateral guide cannulas aimed at the lateral ventricles. Starting 10 days after surgery, mice received a series of bilateral 1 ul intraventricular (i.vent.) injections of an opiate agonist (either [D-Pen2, D-Pen5] Enkephalin at doses of 2.5, 5 or 15ug [DPDPE]; or 30ug of morphine sulfate) or saline. This initial injection was followed, 30 minutes later, by a similar i.vent. injection of a delta receptor antagonist (ICI 174,864/04 at doses of 1 or 10ug [ICI]) or saline. Activity was measured (using the Omnitech Digiscan system) in the time period between the 2 injections and then for 1 hour after the second injection. Mice received multiple drug treatments in randomized order (yielding an N = 6+/group). To provide a dose-response comparison, other mice (N=6/group) received a single injection of either saline, 1, 5, 10, 20 or 30 mg/kg morphine sulfate, i.p., followed by 1 hour of locomotor activity recording.

DPDPE induced a dose-dependant locomotor hyperactivity characterized by the rapid onset of increased horizontal movements interspersed with rearing. In contrast, the intracranial morphine-induced locomotor activation was slower to develop and consisted of less vertical activity. Peripherally administered morphine produced dose-dependant horizontal movements that were not consistently correlated (positively or negatively) with the rearing recorded simultaneously. Administration of the delta receptor antagonist (ICI) reduced the DPDPE-induced hyperactivity but failed to significantly challenge morphine-induced locomotion.

These data suggest that locomotion can be stimulated by either a opioid delta receptor agonist or morphine. However, our findings reveal a partial dissimilarity in the topography of the hyperactivity produced by morphine and that observed after DPDPE. Further, the fact that ICI did not reverse morphine hyperactivity suggests that delta opioid receptors play a very limited role in the morphine-induced locomotion of this mouse.

476.12 MORPHINE WITHDRAWAL: MODULATION OF AGGRESSION AND MOTORIC ACTIVITY AT D1 AND D2 RECEPTORS. K.A. Miczek and J.W. Mohazab*. Dept. of Psychology, Tufts University, Medford, MA 02155.

Substantial and complex neurochemical, physiological and behavioral changes result from chronic exposure to opiates and subsequent withdrawal. Of particular importance appear to be dopamine-mediated functions such as explosive motor responses and profound disturbances in autonomic homeostasis. The objective of our studies was to determine whether or not and, if so, how activation of D1 and D2 receptors selectively modulates certain symptoms of morphine withdrawal, with special attention to heightened aggressive behavior.

Several experiments were designed with mice that were housed as male-female pairs. Before subcutaneous implantation with either placebo or 75-mg morphine pellets, we ascertained that the males exhibited aggressive behavior toward an intruder into their home cage, and we measured their baseline response to pain and rectal temperature. After 3 days of exposure, the nylon-mesh bag containing the subcutaneous pellet was removed. Five hours after pellet removal physiological and behavioral tests were conducted. This time course was determined previously by measuring the peak frequency of jumps and heightened aggressive behavior as critical indices of morphine withdrawal. During withdrawal from either morphine or placebo pellets, mice were assigned to the following pharmacological challenges: (1) Quinpirole (LY171555) at either 0.1, 0.3, 0.6, or 1.0 mg/kg, (2) SKF38393 at either 10, 30, 56, or 100 mg/kg, (3) Combined treatment with quinpirole and SKF38393. Changes in rectal temperature, tail flick response to a radiant heat stimulus, motor activity (walking, rearing, grooming), and aggressive behavior behavior toward and intruder male were measured. Videorecords of 5-min observation periods for motor activity and aggressive behavior were analyzed with the aid of a computerized data acquisition system.

Withdrawal from morphine (1) increased attack bites and threats, (2) disrupted walking and rearing while increasing self-directed grooming, (3) lowered body temperature, and (4) led to explosive motor behavior. In placebo mice, SKF38393 decreased aggressive behavior at doses that did not impair motor activity nor body temperature. By contrast, quinpirole markedly suppressed aggressive and motor behavior and led to hypothermia. In morphine-withdrawn animals, the behavioral and physiological effects of both D1 and D2 agonists were significantly attenuated. If given in combination, both drugs shift the distribution of the motoric elements in the behavioral repertoire of the animals.

476.13 VOLITIONAL DRINKING OF OPIATE SOLUTIONS BY RATS: STUDIES USING A NOVEL PRESENTATION DEVICE. K.R. Carlson. Pharmacology Dept., U. Massachusetts Medical School, Worcester, MA 01655.

A paradigm of oral self-administration is presented which meets the following criteria: 1) It is truly volitional, in that the Ss have a choice between drug and vehicle, 2) The choice is presented in a way which negates rats' position preferences, allowing a meaningful comparison of intakes, 3) The influence of opiates' bitter taste is eliminated, without masking it with sucrose, 4) The intake of opiate increases over time as tolerance develops, and 5) Rats consume enough to become physically dependent.

Ss are housed individually in Plexiglas cages with a 1 cm diameter hole in one end wall. The presentation device consists of a horizontal platform resting on a pivot. Two metal drinking tubes are attached 2.5 cm apart and projecting past one end of the platform. They are connected by flexible tubing to glass burettes suspended above the platform. A vertical post projects up from the other end of the platform. Behind it a 1/4 RPM timing motor is mounted such that as it turns, an arm contacts the post, first near the top, then with a half-revolution near the bottom. As it moves the post, the platform swivels horizontally until it reaches a stopper post attached to the base, placing the end of a tube about 3 mm behind the hole in the cage wall. As the motor continues to turn, the arm reaches the post again, swiveling the platform back and putting the other tube within reach. Thus, the tube which is available regularly alternates every 2 min., the transition taking 10-15 sec. A microswitch mounted on one of the stopping posts signals to the recording device which tube is available. A drinkometer circuit registers each lick. Ss quickly learn to use the apparatus and to switch smoothly between tubes. Each day's activity at each tube is reduced to: A) total number of licks, B) mean number of licks in a bout, i.e., a series of licks terminated by S or by the tube moving out of position, C) mean number of licks after switching from one tube to the other, and D) total number of bouts.

The high potency of fentanyl citrate allows it to be diluted to apparent tastelessness. After a baseline water-water choice period, rats were given a fentanyl-water choice, 6 days apiece at 10, 20, 30, and 40 µg/ml. Drug could not be distinguished from vehicle, in that total licks, mean licks/bout, and mean licks after switching tubes were equal for the two tubes. Total licks for both solutions rose dramatically during exposure to the higher concentrations, indicating that tolerance developed. When a water-water choice was reinstated, Ss lost an average of 3% of their body weight, indicating that a moderate level of physical dependence had been established.

In control studies, morphine (30-500 µg/ml) and levorphanol (3-400 µ/ml) were distinguished from water and were not consumed.

476.14 INHIBITION OF ETHANOL-INDUCED LOCOMOTION IN MICE. R. J. Douglas,* E. T. McGarvey,* C. Reilly,* (SPON: J. B. Simpson) Psychology Dept., Univ. of Washington, Seattle, WA 98195

Mice were run in a maze with a choice of two circular paths. The runs were monitored by breaks in photoelectric beams and recorded on an Esterline Angus. Mice increase running behavior immediately after an injection of ethanol. Other drugs have been shown to potentiate or inhibit the locomotion stimulating effects of alcohol. Catecholaminergic stimulants increase general activity but detract from alcohol induced running. Drugs affecting the cholinergic system also modify this running, the inhibitor scopalamine potentiating it, and the stimulant physostigmine blocking it.

This study addressed the possibility of serotonergic involvement. A saturated solution of tryptophan in distilled water was injected i.p. at .24 gm. tryptophan per kg. body weight. Thirty minutes later the mouse received an ethanol injection i.p. at 1.25 gm. ethanol per kg. body weight. The dose was diluted: 90% saline, 10% ethanol. The mouse was immediately put into the maze to run for half an hour. The mean number of circuits completed after alcohol was $\bar{x} = 86.7$. After pre-administration of tryptophan, $\bar{x} = 56.2$. ($n = 9$, $p < .05$) For comparison, the mean number of circuits without drugs was 23.8; with saline injection, 21.7; with tryptophan and no alcohol, 24.3.

Previously, a 25% solution of polyethylene glycol (PEG) in saline also had been found to block ethanol-induced running. (NeuroSci. Abst. 283.6, 1984) That finding was replicated. ($\bar{x} = 86.7$ without PEG; $\bar{x} = 37.4$ with PEG, $n = 9$, $p < .01$) Further, when PEG and tryptophan were administered together prior to the ethanol injection, a significan interaction was seen. ($\bar{x} = 19.1$, $p < .01$)

476.15 ETHANOL ANTAGONIST Ro 15-4513 IS NOT SELECTIVE FOR ETHANOL. K. T. Britton, L. Percy* and G. F. Koob. VA Med. Center and UCSD Medical School and Alcohol Research Center, SCRF, La Jolla, CA 92037.

Ro 15-4513, an analogue of the benzodiazepine receptor antagonist Ro 15-1788, has been reported to selectively block the anxiolytic and intoxicating properties of ethanol (Suzdak, et al, Science, 234, 1243, 1986). We tested Ro 15-4513 in an operant conflict paradigm sensitive to alcohol effects. Ro 15-4513 (0, 1.5, 3.0, 6.0 mg/kg) produced a significant decrease in both punished and unpunished responding in the conflict test. Ethanol (0.75 g/kg) produced a significant release of punished responding that was blocked by pretreatment with 6.0 mg/kg Ro 15-4513. Ro 15-4513 similarly blocked the anticonflict action of pentobarbitol (4 mg/kg) and chlordiazepoxide (5 mg/kg).

Rats were also trained on an operant reaction time task involving holding down a lever for 0.25-2.0 seconds to obtain food. Animals treated with lg/kg of ethanol or 7.5 mg/kg of pentobarbital showed a significant disruption in performance. This disruptive effect was reversed by Ro 15-4513 and FG 7142 in doses of 1.5-6.0 mg/kg. These results suggest that Ro 15-4513 shares many of the inverse agonist properties of the beta carboline FG 7142, and that this inverse agonist activity is the basis for its antagonistic effects against ethanol.

476.16 **ETHANOL ANTAGONIST RO 15-4513: ANXIOGENIC EFFECT DEMONSTRATED IN PENTYLENETETRAZOL DISCRIMINATION.** **C.M. Harris, D. Benjamin*, and H. Lal, Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107**

The imidazobenzodiazepine Ro 15-4513, proposed as a selective ethanol antagonist, blocks the anxiolytic action of ethanol (Suzdak et al., Science 234:1243-1247), and produces several direct behavioral and physiological effects opposite to those of anxiolytics. (Bonetti et al., Neurosci. Lett. Suppl. 18:30, 1984; Miczek and Weerts, Science 235:1127). To test the hypothesis that Ro 15-4513 has a direct anxiogenic action, the drug was tested in the pentylenetetrazol (PTZ) discrimination, a bioassay for anxiogenic drugs. This approach is based on observations that anxiogenic drugs mimick and anxiolytic drugs block the discriminative stimulus produced by PTZ (for description and review, see Lal and Fielding, Drug Dev. Res. 4:3-21, 1984). Rats were trained in a 2-lever food-reinforced operant task to press one lever after injection of PTZ, and the other lever after saline. They were then utilized in drug tests in which the percentage of rats selecting the PTZ-appropriate lever served as a measure of the intensity of PTZ-like stimuli. Rats selected the PTZ-lever in a dose-dependent manner after Ro 15-4513 (ED50 = 2.1 mg/kg), indicating a PTZ-like subjective effect. Behavioral toxicity was not evident; convulsions, tremors, or rigidity did not occur at doses of Ro 15-4513 up to 40 mg/kg, and, the % rats completing the lever-pressing task was not significantly reduced at any dose. The PTZ-like stimulus of Ro 15-4513 was antagonized in a dose-dependent manner by pretreatment with the anxiolytic drug diazepam (ED50 = 9.0 mg/kg), further indicating the PTZ-like quality of the Ro 15-4513 stimulus. The PTZ-like stimulus produced by Ro 15-4513 was blocked by the benzodiazepine receptor antagonist Ro 15-1788. These data support the hypothesis that Ro 15-4513 is anxiogenic and that its effect is mediated by its action at the benzodiazepine receptor. (Ro 15-4513, Ro 15-1788 and diazepam were supplied free of charge by the Hoffmann-La Roche Company; the study was supported by grant # RO1-AA06890 from NIAAA.)