## Contents—Part 2

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Program Committee</td>
<td>iv</td>
</tr>
<tr>
<td>Chronological List of Sessions</td>
<td>v</td>
</tr>
<tr>
<td>Thematic List of Sessions</td>
<td>xii</td>
</tr>
<tr>
<td>Abstracts in Session Order*</td>
<td></td>
</tr>
<tr>
<td>Wednesday, October 31-Friday, November 2</td>
<td>907</td>
</tr>
<tr>
<td>Key Word Index</td>
<td>1,352</td>
</tr>
<tr>
<td>Author Index</td>
<td>1,590</td>
</tr>
</tbody>
</table>

*7,979 volunteer abstracts, 15 symposia abstracts.
1990 Program Committee

Edward G. Jones, M.D., Ph.D., Chairperson
University of California College of Medicine

J. Anthony Movshon, Ph.D.
Incoming Chairperson
New York University

Richard W. Aldrich, Ph.D.
Stanford University Medical School

Theodore W. Berger, Ph.D.
University of Pittsburgh

James E. Blankenship, Ph.D.
Marine Biomedical Institute

Dennis W. Choi, M.D., Ph.D.
Stanford Medical School

Roland Ciaranello, M.D.
Stanford University Medical Center

Suzanne H. Corkin, Ph.D.
Massachusetts Institute of Technology

Antonio R. Damasio, M.D., Ph.D.
University of Iowa College of Medicine

Robert P. Elde, Ph.D.
University of Minnesota

Eberhard E. Fetz, Ph.D.
University of Washington School of Medicine

Howard L. Fields, M.D., Ph.D.
University of California Medical School

Eric Frank, Ph.D.
University of Pittsburgh Medical School

Karl Herrup, Ph.D.
Eunice Kennedy Shriver Center

Stephen M. Highstein, M.D., Ph.D.
Washington University School of Medicine

John P. Horn, Ph.D.
University of Pittsburgh Medical School

Robert L. Macdonald, M.D., Ph.D.
University of Michigan

Urs S. Rutishauser, Ph.D.
Case Western Reserve University

Elaine Sanders-Bush, Ph.D.
Vanderbilt University School of Medicine

Gerard P. Smith, M.D.
Cornell Medical Center

C. Dominique Toran-Allerand, M.D.
Columbia University College of Physicians and Surgeons

Wylie W. Vale, Ph.D.
The Salk Institute

Robert D. Wurster, Ph.D.
Loyola University of Chicago

Patricia Goldman-Rakic, Ph.D., ex officio
Yale University School of Medicine

Robert H. Wurtz, Ph.D., ex officio
National Institutes of Health

Larry R. Squire, Ph.D., ex officio
University of California, San Diego Medical School
Session Number and Title Page

SUNDAY

Animal Care Tutorial—5:30 p.m.
1. Psychological Well-Being of Laboratory Primates: Regulations and Reality No abstract
Public Lecture—8:00 p.m.
2. Limbic-Cortical Neural Circuits and the Pathophysiology of Schizophrenia, D.R. Weinberger No abstract

MONDAY

Symposia—8:30 a.m.
3. Molecular Mechanisms of Neuronal Guidance
   Chaired by: M.E. Hatten 1
4. Regulation of Pattern Generating Networks
   Chaired by: K.G. Pearson 1

Slide Sessions—8:30 a.m.
5. Cellular and molecular biology of catecholamine receptors 1
6. Potassium channels 3
7. Sensory systems—visual cortex: motion pathways 5
8. Peptides—receptors 7
9. Acetylcholine—receptors 9
10. Cardiovascular regulation I 11
11. Drugs of abuse: cocaine 13
12. Alzheimer’s disease: amyloid I 15
13. Ischemia I 17
14. Invertebrate learning and behavior I 19
15. Epilepsy: basic mechanisms I 21
16. Brain metabolism and blood flow: methods 23
17. Chemical senses: peripheral mechanisms I 25
18. Human behavioral neurobiology 26

Poster Sessions—8:30 a.m.
19. The aging process: neurotransmitters and endocrine regulation 28
20. Substances of abuse 32
21. Nutritional and prenatal factors 34
22. Transplantation: general I 36
23. Transplantation: new techniques and cell lines 38
24. Synaptogenesis in the CNS I 41
25. Neuronal plasticity in adult animals I 42
26. Blood-brain barrier 45
27. Cytoskeleton, transport and membrane targeting 49
28. Staining, tracing and imaging techniques I 52
29. Staining, tracing and imaging techniques II 53
30. Postsynaptic mechanisms I 56
31. Synaptic transmission 59
32. Neurochemistry of transmitter systems 62

Session Number and Title Page

33. Other biogenic amines and purines 65
34. Catecholamines I 68
35. Serotonin I 72
36. GABA?, receptors I 75
37. Dopamine physiology I 79
38. Peptides—receptors: CCK, somatostatin 82
39. Excitatory amino acids: receptors I 85
40. Excitatory amino acids: receptors II 88
41. Hypothalamic-pituitary-adrenal regulation: CRF 91
42. Neuroendocrine regulation: other I 93
43. Pain modulation: anatomy and physiology I 96
44. Pain modulation: anatomy and physiology II 99
45. Chemical senses: central pathways I 101
46. Sensory systems—visual psychophysics and behavior I 104
47. Sensory systems—subcortical visual pathways: superior colliculus and related 108
48. Spinal cord and brainstem 110
49. Spinal cord and brainstem: motoneurons 113
50. Control of posture and movement I 115
51. Muscle: molecular studies 118
52. Muscle: general 119
53. Hippocampus and amygdala: neuroanatomy 121
54. Limbic system I 124
55. Comparative neuroanatomy: fish and amphibia 126
56. Monoamines and behavior I 130
57. Drugs of abuse: alcohol I 133
58. Learning and memory—pharmacology: acetylcholine I 136
59. Mental illness: depression, suicide, other 139
60. Neuromuscular diseases 141

Presidential Special Lecture—11:45 a.m.
61. Exciting Currents at Hippocampal Synapses: EPSPs, EPSCs and LTPs
   R.A. Nicoll No abstract

Symposium—1:00 p.m.
62. Hair Cells of the Inner Ear: Structure, Transduction, and Active Motion
   Chaired by: D.P. Corey 143

Special Lecture—1:00 p.m.
63. Excitement, Fos, and Fitts: The Role of NMDA Receptors and Early Genes in Kindling
   J.O. McNamara No abstract

Special Lecture—2:30 p.m.
64. Strategies for Understanding Gene Defects in Neurologic Diseases
   R. Davis No abstract

Slide Sessions—1:00 p.m.
65. Long-term potentiation I 144
66. Stress, hormones and the autonomic nervous system I 146
<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>67. Alzheimer’s disease: cognitive and clinical studies</td>
<td>148</td>
</tr>
<tr>
<td>68. Process outgrowth, growth cones and guidance mechanisms I</td>
<td>150</td>
</tr>
<tr>
<td>69. Control of posture and movement II</td>
<td>152</td>
</tr>
<tr>
<td>70. Nerve growth factors I</td>
<td>154</td>
</tr>
<tr>
<td>71. Gene structure and function I</td>
<td>156</td>
</tr>
<tr>
<td>72. Sensory systems—subcortical visual pathways: LGN .</td>
<td>158</td>
</tr>
<tr>
<td>73. Pain modulation: anatomy and physiology III</td>
<td>160</td>
</tr>
<tr>
<td>74. Regeneration: general studies and molecular correlates</td>
<td>162</td>
</tr>
<tr>
<td>75. Monoamines and behavior II</td>
<td>164</td>
</tr>
<tr>
<td>76. Presynaptic mechanisms I</td>
<td>166</td>
</tr>
</tbody>
</table>

**Poster Sessions—1:00 p.m.**

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>77. Regeneration: genes, inhibitory factors and axonal transport</td>
<td>168</td>
</tr>
<tr>
<td>78. Neuronal death: mechanisms</td>
<td>170</td>
</tr>
<tr>
<td>79. Cell lineage I</td>
<td>173</td>
</tr>
<tr>
<td>80. Differentiation, morphogenesis and development: glia</td>
<td>175</td>
</tr>
<tr>
<td>81. Differentiation, morphogenesis and development: currents, channels, muscle development</td>
<td>177</td>
</tr>
<tr>
<td>82. Differentiation, morphogenesis and development: cell surface and matrix components</td>
<td>178</td>
</tr>
<tr>
<td>83. Differentiation, morphogenesis and development: cytoskeleton</td>
<td>179</td>
</tr>
<tr>
<td>84. Sodium channels I</td>
<td>181</td>
</tr>
<tr>
<td>85. Sodium channels II</td>
<td>183</td>
</tr>
<tr>
<td>86. Ion channels: modulation and regulation I</td>
<td>185</td>
</tr>
<tr>
<td>87. Peptides: physiological effects I</td>
<td>188</td>
</tr>
<tr>
<td>88. Excitotoxicity I</td>
<td>190</td>
</tr>
<tr>
<td>89. Excitotoxicity II</td>
<td>193</td>
</tr>
<tr>
<td>90. Excitotoxicity III</td>
<td>197</td>
</tr>
<tr>
<td>91. Acetylcholine I</td>
<td>199</td>
</tr>
<tr>
<td>92. Acetylcholine—receptors: muscarinic I</td>
<td>201</td>
</tr>
<tr>
<td>93. Acetylcholine—receptors: nicotinic I</td>
<td>204</td>
</tr>
<tr>
<td>94. Molecular biology of dopamine receptors</td>
<td>208</td>
</tr>
<tr>
<td>95. Opioids: behavior</td>
<td>210</td>
</tr>
<tr>
<td>96. Brain glutamate systems</td>
<td>213</td>
</tr>
<tr>
<td>97. Cardiovascular regulation: bulbospinal mechanisms</td>
<td>214</td>
</tr>
<tr>
<td>98. Cardiovascular regulation: brainstem and baroreflexes</td>
<td>218</td>
</tr>
<tr>
<td>99. Subcortical somatosensory pathways</td>
<td>221</td>
</tr>
<tr>
<td>100. Somatosensory cortex and thalamocortical relationships I</td>
<td>224</td>
</tr>
<tr>
<td>101. Somatosensory cortex and thalamocortical relationships II</td>
<td>227</td>
</tr>
<tr>
<td>102. Sensory systems—visual cortex: theoretical approaches</td>
<td>229</td>
</tr>
<tr>
<td>103. Somatic and visceral afferents: capsaicin</td>
<td>231</td>
</tr>
<tr>
<td>104. Basal ganglia and thalamus I</td>
<td>232</td>
</tr>
<tr>
<td>105. Basal ganglia and thalamus II</td>
<td>236</td>
</tr>
<tr>
<td>106. Cortex I</td>
<td>240</td>
</tr>
<tr>
<td>107. Cortex II</td>
<td>243</td>
</tr>
<tr>
<td>108. Comparative neuroanatomy: reptiles, birds, mammals</td>
<td>245</td>
</tr>
<tr>
<td>109. Psychotherapeutic drugs: antipsychotics I</td>
<td>247</td>
</tr>
<tr>
<td>110. Drugs of abuse: cocaine, dopamine</td>
<td>251</td>
</tr>
<tr>
<td>111. Drugs of abuse: cocaine, serotonin</td>
<td>254</td>
</tr>
<tr>
<td>112. Drugs of abuse: alcohol II</td>
<td>258</td>
</tr>
<tr>
<td>113. Learning and memory: physiology I</td>
<td>261</td>
</tr>
<tr>
<td>114. Neuropeptides and behavior I</td>
<td>265</td>
</tr>
<tr>
<td>115. Learning and memory: conditioning</td>
<td>268</td>
</tr>
</tbody>
</table>

**Session Number and Title                                                                 | Page |

| 116. Stress, hormones and the autonomic nervous system II                             | 271  |
| 117. Ischemia II                                                                       | 273  |
| 118. Ischemia III                                                                      | 276  |
| 119. Epilepsy: status epilepticus                                                      | 280  |
| 120. Alzheimer’s disease: biochemistry and clinical studies                           | 281  |

**Special Lecture—4:15 p.m.**

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>121. The Neural Organization of Binocular Vision</td>
<td>No abstract</td>
</tr>
<tr>
<td>R.D. Freeman</td>
<td></td>
</tr>
</tbody>
</table>

**Animals in Research Panel—6:30 p.m.**

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>122. Animal Activism 102— Fighting Back in the Public Schools</td>
<td>No abstract</td>
</tr>
</tbody>
</table>

**Presidential Symposium—8:00 p.m.**

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine Receptors and Drug Dependence</td>
<td></td>
</tr>
<tr>
<td>M.J. Kuhar</td>
<td>No abstract</td>
</tr>
<tr>
<td>The Neurobiology of Drug Reinforcement</td>
<td></td>
</tr>
<tr>
<td>R.A. Wise</td>
<td>No abstract</td>
</tr>
<tr>
<td>Addicting Drugs: Neurobiology, Pharmacology, and Policy</td>
<td></td>
</tr>
<tr>
<td>A. Goldstein</td>
<td>No abstract</td>
</tr>
</tbody>
</table>

**Symposia—8:30 a.m.**

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>124. The Olivocerebellar System: Its Possible Role in Learning</td>
<td>283</td>
</tr>
<tr>
<td>Chair: by J.A. Harvey</td>
<td></td>
</tr>
<tr>
<td>125. Direct Ion Channel Gating by Intracellular Ions and Molecules</td>
<td>283</td>
</tr>
<tr>
<td>Chair: by P. Hockberger</td>
<td></td>
</tr>
</tbody>
</table>

**Slide Sessions—8:30 a.m.**

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>126. Hypothalamic-pituitary-gonadal regulation I</td>
<td>284</td>
</tr>
<tr>
<td>127. Learning and human lesion studies</td>
<td>286</td>
</tr>
<tr>
<td>128. Excitotoxicity IV</td>
<td>288</td>
</tr>
<tr>
<td>129. Brain metabolism and blood flow: central influences</td>
<td>290</td>
</tr>
<tr>
<td>130. Sensory systems—visual cortex: architecture and function</td>
<td>292</td>
</tr>
<tr>
<td>131. Digestive behavior: monoamines and nutrients</td>
<td>294</td>
</tr>
<tr>
<td>132. Nerve growth factors II</td>
<td>296</td>
</tr>
<tr>
<td>133. Cardiovascular regulation II</td>
<td>298</td>
</tr>
<tr>
<td>134. Interactions between neurotransmitters I</td>
<td>300</td>
</tr>
<tr>
<td>135. Cellular and molecular studies I</td>
<td>301</td>
</tr>
<tr>
<td>136. Drugs of abuse: amphetamine and cocaine</td>
<td>303</td>
</tr>
<tr>
<td>137. Transmitters in invertebrates I</td>
<td>305</td>
</tr>
<tr>
<td>138. Epilepsy: human studies and animal models</td>
<td>307</td>
</tr>
</tbody>
</table>

**Poster Sessions—8:30 a.m.**

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>139. Process outgrowth, growth cones and guidance mechanisms II</td>
<td>309</td>
</tr>
<tr>
<td>140. Process outgrowth, growth cones and guidance mechanisms III</td>
<td>312</td>
</tr>
<tr>
<td>141. Differentiation, morphogenesis and development: fiber guidance and synaptogenesis</td>
<td>316</td>
</tr>
<tr>
<td>142. Specificity of synaptic connections</td>
<td>317</td>
</tr>
</tbody>
</table>

**TUESDAY**

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>124. The Olivocerebellar System: Its Possible Role in Learning</td>
<td>283</td>
</tr>
<tr>
<td>Chair: by J.A. Harvey</td>
<td></td>
</tr>
<tr>
<td>125. Direct Ion Channel Gating by Intracellular Ions and Molecules</td>
<td>283</td>
</tr>
<tr>
<td>Chair: by P. Hockberger</td>
<td></td>
</tr>
</tbody>
</table>

**Symposia—8:30 a.m.**

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>126. Hypothalamic-pituitary-gonadal regulation I</td>
<td>284</td>
</tr>
<tr>
<td>127. Learning and human lesion studies</td>
<td>286</td>
</tr>
<tr>
<td>128. Excitotoxicity IV</td>
<td>288</td>
</tr>
<tr>
<td>129. Brain metabolism and blood flow: central influences</td>
<td>290</td>
</tr>
<tr>
<td>130. Sensory systems—visual cortex: architecture and function</td>
<td>292</td>
</tr>
<tr>
<td>131. Digestive behavior: monoamines and nutrients</td>
<td>294</td>
</tr>
<tr>
<td>132. Nerve growth factors II</td>
<td>296</td>
</tr>
<tr>
<td>133. Cardiovascular regulation II</td>
<td>298</td>
</tr>
<tr>
<td>134. Interactions between neurotransmitters I</td>
<td>300</td>
</tr>
<tr>
<td>135. Cellular and molecular studies I</td>
<td>301</td>
</tr>
<tr>
<td>136. Drugs of abuse: amphetamine and cocaine</td>
<td>303</td>
</tr>
<tr>
<td>137. Transmitters in invertebrates I</td>
<td>305</td>
</tr>
<tr>
<td>138. Epilepsy: human studies and animal models</td>
<td>307</td>
</tr>
</tbody>
</table>

**Poster Sessions—8:30 a.m.**

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>139. Process outgrowth, growth cones and guidance mechanisms II</td>
<td>309</td>
</tr>
<tr>
<td>140. Process outgrowth, growth cones and guidance mechanisms III</td>
<td>312</td>
</tr>
<tr>
<td>141. Differentiation, morphogenesis and development: fiber guidance and synaptogenesis</td>
<td>316</td>
</tr>
<tr>
<td>142. Specificity of synaptic connections</td>
<td>317</td>
</tr>
<tr>
<td>Session Number and Title</td>
<td>Page</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------</td>
</tr>
<tr>
<td>143. Endocrine control and development I</td>
<td>320</td>
</tr>
<tr>
<td>144. Endocrine control and development II</td>
<td>325</td>
</tr>
<tr>
<td>145. Motor systems: development and plasticity I</td>
<td>329</td>
</tr>
<tr>
<td>146. Motor systems: development and plasticity II</td>
<td>332</td>
</tr>
<tr>
<td>147. Development and plasticity—visual system: retina and optic nerve</td>
<td>333</td>
</tr>
<tr>
<td>148. Regeneration: molecular correlates</td>
<td>337</td>
</tr>
<tr>
<td>149. Ontogeny of dopaminergic systems</td>
<td>340</td>
</tr>
<tr>
<td>150. Neural plasticity in adult animals II</td>
<td>341</td>
</tr>
<tr>
<td>151. mRNA regulation: general</td>
<td>344</td>
</tr>
<tr>
<td>152. Staining, tracing and imaging techniques III</td>
<td>346</td>
</tr>
<tr>
<td>153. Neuroglia and myelin I</td>
<td>349</td>
</tr>
<tr>
<td>154. Gene structure and function II</td>
<td>353</td>
</tr>
<tr>
<td>155. Ion channels: modulation and regulation II</td>
<td>355</td>
</tr>
<tr>
<td>156. Potassium channels II</td>
<td>359</td>
</tr>
<tr>
<td>157. Peptides: anatomical localization I</td>
<td>361</td>
</tr>
<tr>
<td>158. Opioids: receptors I</td>
<td>365</td>
</tr>
<tr>
<td>159. Opioids: receptors II</td>
<td>368</td>
</tr>
<tr>
<td>160. Second messengers I</td>
<td>371</td>
</tr>
<tr>
<td>161. Excitatory amino acids: pharmacology I</td>
<td>375</td>
</tr>
<tr>
<td>162. GABA receptors II</td>
<td>378</td>
</tr>
<tr>
<td>163. Regulation of catecholamine receptors</td>
<td>381</td>
</tr>
<tr>
<td>164. Cell biology of catecholamine receptors</td>
<td>383</td>
</tr>
<tr>
<td>165. Behavioral pharmacology: acetylcholine, monoamines, and benzodiazepines</td>
<td>387</td>
</tr>
<tr>
<td>166. Peptides: biosynthesis and metabolism I</td>
<td>390</td>
</tr>
<tr>
<td>167. Hypothalamic-pituitary-gonadal regulation: modulation by peptides</td>
<td>393</td>
</tr>
<tr>
<td>168. Hypothalamic-pituitary-gonadal regulation: GnRH</td>
<td>396</td>
</tr>
<tr>
<td>169. Invertebrate sensory systems</td>
<td>399</td>
</tr>
<tr>
<td>170. Chemical senses: central pathways II</td>
<td>403</td>
</tr>
<tr>
<td>171. Sensory systems—retina: receptors, outer retina, ERG</td>
<td>405</td>
</tr>
<tr>
<td>172. Pain modulation: spinal opioid pharmacology</td>
<td>409</td>
</tr>
<tr>
<td>173. Pain modulation: pharmacology I</td>
<td>410</td>
</tr>
<tr>
<td>174. Spinal cord: neurotransmitters</td>
<td>413</td>
</tr>
<tr>
<td>175. Somatic and visceral afferents I</td>
<td>415</td>
</tr>
<tr>
<td>176. Basal ganglia and thalamus III</td>
<td>417</td>
</tr>
<tr>
<td>177. Muscle: human studies</td>
<td>420</td>
</tr>
<tr>
<td>178. Cortex III</td>
<td>421</td>
</tr>
<tr>
<td>179. Basal ganglia and thalamus IV</td>
<td>425</td>
</tr>
<tr>
<td>180. Basal ganglia and thalamus V</td>
<td>427</td>
</tr>
<tr>
<td>181. Hippocampus and amygdala: neurocytology</td>
<td>428</td>
</tr>
<tr>
<td>182. Stress, hormones and the autonomic nervous system III</td>
<td>432</td>
</tr>
<tr>
<td>183. Drugs of abuse: alcohol III</td>
<td>434</td>
</tr>
<tr>
<td>184. Monoamines and behavior III</td>
<td>436</td>
</tr>
<tr>
<td>185. Learning and memory: physiology II</td>
<td>440</td>
</tr>
<tr>
<td>186. Neuropeptides and behavior II</td>
<td>442</td>
</tr>
<tr>
<td>187. Neurotoxicity: metals</td>
<td>445</td>
</tr>
<tr>
<td>188. Epilepsy: human studies</td>
<td>447</td>
</tr>
<tr>
<td>189. Genetic models of nervous disorders I</td>
<td>449</td>
</tr>
<tr>
<td>190. Alzheimer’s disease: amyloid II</td>
<td>451</td>
</tr>
</tbody>
</table>

**History of Neuroscience Lecture**—11:45 a.m.

**Symposium**—1:00 p.m.

**Special Lecture**—1:00 p.m.

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>191. The Good Old Days…Or Were They?</td>
<td>No abstract</td>
</tr>
<tr>
<td>192. Brain Modulation of Sensory Signals</td>
<td>452</td>
</tr>
<tr>
<td>Chair by: R.B. Barlow, Jr.</td>
<td></td>
</tr>
</tbody>
</table>

**Special Lecture**—2:30 p.m.

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>193. Embryonic Induction and Axial Patterning in Vertebrates</td>
<td>D.A. Melton</td>
</tr>
</tbody>
</table>

**Slide Sessions**—1:00 p.m.

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>195. Hypothalamic-pituitary-adrenal regulation</td>
<td>453</td>
</tr>
<tr>
<td>196. Formation and maturation of synaptic connections</td>
<td>455</td>
</tr>
<tr>
<td>197. Process outgrowth, growth cones and guidance mechanisms IV</td>
<td>457</td>
</tr>
<tr>
<td>198. Drugs of abuse: alcohol IV</td>
<td>459</td>
</tr>
<tr>
<td>199. Alzheimer’s disease: neuropathology I</td>
<td>460</td>
</tr>
<tr>
<td>200. Excitatory amino acids: pharmacology II</td>
<td>462</td>
</tr>
<tr>
<td>201. Sensory systems—retina: retinal circuits</td>
<td>465</td>
</tr>
<tr>
<td>202. Postsynaptic mechanisms II</td>
<td>467</td>
</tr>
<tr>
<td>203. Transplantation: general II</td>
<td>469</td>
</tr>
<tr>
<td>204. Hormonal control of behavior I</td>
<td>471</td>
</tr>
<tr>
<td>205. Learning and memory: physiology III</td>
<td>473</td>
</tr>
<tr>
<td>206. Trauma</td>
<td>475</td>
</tr>
</tbody>
</table>

**Poster Sessions**—1:00 p.m.

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>207. Nerve growth factors III</td>
<td>476</td>
</tr>
<tr>
<td>208. Nerve growth factors IV</td>
<td>480</td>
</tr>
<tr>
<td>209. Other trophic agents I</td>
<td>483</td>
</tr>
<tr>
<td>210. Transplantation: anatomical projections</td>
<td>487</td>
</tr>
<tr>
<td>211. Regeneration: specificity and functional recovery</td>
<td>488</td>
</tr>
<tr>
<td>212. Long-term potentiation II</td>
<td>490</td>
</tr>
<tr>
<td>213. Development and plasticity—visual system: cortical anatomy</td>
<td>493</td>
</tr>
<tr>
<td>214. Membrane composition and cell surface macromolecules I</td>
<td>496</td>
</tr>
<tr>
<td>215. Synaptic structure and function I</td>
<td>499</td>
</tr>
<tr>
<td>216. Presynaptic mechanisms II</td>
<td>501</td>
</tr>
<tr>
<td>217. Presynaptic mechanisms III</td>
<td>503</td>
</tr>
<tr>
<td>218. Potassium channels III</td>
<td>506</td>
</tr>
<tr>
<td>219. Calcium channels: pharmacology</td>
<td>509</td>
</tr>
<tr>
<td>220. Peptides—receptors: other</td>
<td>513</td>
</tr>
<tr>
<td>221. Peptides: physiological effects II</td>
<td>516</td>
</tr>
<tr>
<td>222. Peptides: anatomical localization II</td>
<td>519</td>
</tr>
<tr>
<td>223. Interactions between neurotransmitters II</td>
<td>521</td>
</tr>
<tr>
<td>224. Catecholamines II</td>
<td>524</td>
</tr>
<tr>
<td>225. Serotonin II</td>
<td>529</td>
</tr>
<tr>
<td>226. Dopamine physiology II</td>
<td>531</td>
</tr>
<tr>
<td>227. Acetylcholine—receptors: muscarinic II</td>
<td>535</td>
</tr>
<tr>
<td>228. Second messengers II</td>
<td>537</td>
</tr>
<tr>
<td>229. Excitatory amino acids: receptors III</td>
<td>540</td>
</tr>
<tr>
<td>230. Excitatory amino acids: receptors IV</td>
<td>542</td>
</tr>
<tr>
<td>231. Excitatory amino acids: receptors V</td>
<td>545</td>
</tr>
<tr>
<td>232. Transmitters in invertebrates II</td>
<td>548</td>
</tr>
<tr>
<td>233. Cardiovascular regulation: brainstem mechanisms</td>
<td>552</td>
</tr>
<tr>
<td>234. Cardiovascular regulation: hypothalamic, limbic, and cortical mechanisms</td>
<td>556</td>
</tr>
<tr>
<td>235. Spinal cord: anatomy and physiology</td>
<td>560</td>
</tr>
<tr>
<td>236. Pain modulation: anatomy and physiology IV</td>
<td>562</td>
</tr>
<tr>
<td>237. Pain modulation: pharmacology II</td>
<td>564</td>
</tr>
<tr>
<td>238. Sensory systems—visual cortex: evoked potentials and stimulation</td>
<td>568</td>
</tr>
<tr>
<td>239. Brain metabolism and blood flow: endogenous factors</td>
<td>571</td>
</tr>
</tbody>
</table>
Session
Number and Title.......................................................... Page
240. Hypothalamus ...................................................... 573
241. Human behavioral neurobiology: event related
potentials, attention, audition ...................................... 577
242. Drugs of abuse: cocaine and others ...................... 580
243. Drugs of abuse: amphetamine ............................... 583
244. Psychotherapeutic drugs: antipsychotics II .......... 587
245. Motivation and self-stimulation .............................. 590
246. Invertebrate learning and behavior II ................. 594
247. Motivation and emotion ......................................... 598
248. Biological rhythms and sleep I ............................... 601
249. Learning and memory: anatomy I ......................... 604
250. Learning and memory: anatomy II ....................... 607
251. Neurotoxicity: PNS and retina .............................. 609
252. Alzheimer’s disease: pharmacology ..................... 611
253. Infectious diseases ............................................... 614

Special Lecture—4:15 p.m.
254. The Glutamate Receptors: Genes, Structure, Function and
Expression
S. Heinemann .................................................................. No abstract

Grass Foundation Lecture—8:00 p.m.
255. A Constructionist View of Postnatal Brain Development
D. Purves ....................................................................... No abstract

WEDNESDAY

Symposia—8:30 a.m.
256. Recapitulation of Developmental Mechanisms in
Neurodegenerative Disorders
Chair ed by: M.P. Mattson ................................. 615
257. Galanin: Multidisciplinary Studies
Chair ed by: S.F. Leibowitz ........................................ 615

Slide Sessions—8:30 a.m.
258. Learning and memory: non-human primate lesion
studies ................................................................. 616
259. Excitatory amino acids: receptors VI .................. 618
260. Sensory systems—visual cortex: extrastriate cortex .... 620
261. Calcium channels I ............................................. 622
262. Process output growth, growth cones and guidance
mechanisms V ..................................................... 624
263. Invertebrate learning and behavior III .............. 626
264. Second messengers III ......................................... 628
265. Sensory systems—development and plasticity I .... 630
266. Transmitters in invertebrates III ......................... 632
267. Pain modulation: pharmacology III .................... 634
268. Motor systems and sensorimotor integration:
cerebellum I .......................................................... 636
269. Regulation of autonomic and respiratory functions .... 638
270. Biological rhythms and sleep II ......................... 640

Poster Sessions—8:30 a.m.
271. Differentiation, morphogenesis and development:
molecular correlates ................................................. 642
272. Differentiation, morphogenesis and development:
neurotransmitters, peptides, hormones .................... 644
273. Cell lineage II ................................................... 648
274. Long-term potentiation III ................................. 651
275. Transplantation: behavioral and electrophysiological
effects .............................................................. 654

Session
Number and Title.......................................................... Page
276. Neural plasticity in adult animals III .................... 657
277. mRNA regulation: endocrine connection ............. 660
278. Gene structure and function III .......................... 662
279. Neuroglia and myelin II ...................................... 664
280. Ion channels: chloride and other channels .......... 668
281. Potassium channels IV ........................................ 670
282. Synaptic structure and function II ...................... 673
283. Calcium channels II ............................................ 675
284. Excitatory amino acids: pharmacology III .......... 679
285. Acetylcholine—receptors: nicotinic II ................ 681
286. Peptides—receptors: angiotensin, endothelin ........ 684
287. Mechanisms in transporter physiology ............... 686
288. Localization of neurotransmitter receptors I ........ 688
289. GABA receptors III ............................................ 691
290. Localization of SHT receptor subtypes ................. 694
291. Localization of neurotransmitter receptors II ........ 695
292. Hypothalamic-pituitary-adrenal regulation: other .... 698
293. Somatic and visceral afferents II ......................... 701
294. Pain: pathways I ............................................... 703
295. Sensory systems—visual cortex: connections ........ 707
296. Sensory systems—subcortical visual pathways:
retinal projections and thalamus ............................... 710
297. Sensory systems—retina: retinal signals ............... 712
298. Sensory systems—auditory system:
central pathways I ................................................. 714
299. Sensory systems—auditory system:
central physiology I ............................................... 718
300. Sensory systems—auditory system structure:
fundation of identified cells ...................................... 721
301. Circuitry and pattern generation I ....................... 724
302. Spinal cord and brainstem: pharmacological
studies ...................................................................... 727
303. Spinal cord and brainstem: anatomy .................... 729
304. Motor systems and sensorimotor integration:
vestibular system I .................................................. 732
305. Motor systems and sensorimotor integration:
vestibular system II ................................................. 734
306. Hippocampus and amygdala: neuropsychology I .... 736
307. Brain metabolism and blood flow: exogenous
factors ........................................................................ 739
308. Hormonal control of behavior II ......................... 741
309. Drugs of abuse: cocaine, cellular ......................... 745
310. Drugs of abuse: cocaine, behavior ....................... 748
311. Monoamines and behavior IV ............................. 751
312. Drugs of abuse: alcohol V .................................... 754
313. Neuroethology: invertebrates .............................. 757
314. Learning and memory: physiology IV .................. 760
315. Hormonal control of behavior III ....................... 763
316. Learning and memory—pharmacology: excitatory
amino acids .......................................................... 766
317. Biological rhythms and sleep III ......................... 769
318. Ingestive behavior: peptides I ............................. 773
319. Trauma: brain injury ............................................ 777
320. Epilepsy: animal genetic models ......................... 780
321. Epilepsy: anti-convulsant drugs .......................... 782
322. Alzheimer’s disease: amyloid III ....................... 786
323. Disorders of the nervous system: developmental
models ........................................................................ 788

Warner-Lambert Lecture—11:45 a.m.
324. Excitatory Synaptic Control of Hippocampal Neurons
P. Andersen ................................................................ No abstract
### Session Number and Title

<table>
<thead>
<tr>
<th>Session</th>
<th>Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>399.</td>
<td>Transplantation: new techniques, immune rejection and behavior</td>
<td>963</td>
</tr>
<tr>
<td>400.</td>
<td>Second messengers IV</td>
<td>966</td>
</tr>
<tr>
<td>401.</td>
<td>Motor systems and sensorimotor integration: vestibular system III</td>
<td>968</td>
</tr>
<tr>
<td>402.</td>
<td>Neuroglia and myelin III</td>
<td>969</td>
</tr>
<tr>
<td>403.</td>
<td>Neural-immune interactions I</td>
<td>971</td>
</tr>
<tr>
<td>404.</td>
<td>Cellular and molecular studies III</td>
<td>973</td>
</tr>
<tr>
<td>405.</td>
<td>Steroids: receptors and actions</td>
<td>975</td>
</tr>
<tr>
<td>406.</td>
<td>Ingestive behavior: peptides II</td>
<td>977</td>
</tr>
</tbody>
</table>

### Poster Sessions—8:30 a.m.

<table>
<thead>
<tr>
<th>Session</th>
<th>Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>407.</td>
<td>Long-term potentiation V</td>
<td>979</td>
</tr>
<tr>
<td>408.</td>
<td>Neuronal death: lesion studies</td>
<td>982</td>
</tr>
<tr>
<td>409.</td>
<td>Development and plasticity—visual system: molecular and cellular mechanisms II</td>
<td>984</td>
</tr>
<tr>
<td>410.</td>
<td>Nerve growth factors VII</td>
<td>987</td>
</tr>
<tr>
<td>411.</td>
<td>Nerve growth factors VIII</td>
<td>990</td>
</tr>
<tr>
<td>412.</td>
<td>Trophic interactions II</td>
<td>993</td>
</tr>
<tr>
<td>413.</td>
<td>Other trophic agents II</td>
<td>996</td>
</tr>
<tr>
<td>414.</td>
<td>Regeneration: general</td>
<td>1001</td>
</tr>
<tr>
<td>415.</td>
<td>Synaptogenesis of neuromuscular junctions</td>
<td>1003</td>
</tr>
<tr>
<td>416.</td>
<td>Process outgrowth, growth cones and guidance mechanisms VIII</td>
<td>1005</td>
</tr>
<tr>
<td>417.</td>
<td>Process outgrowth, growth cones and guidance mechanisms IX</td>
<td>1008</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Session</th>
<th>Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>418.</td>
<td>Membrane composition and cell surface macromolecules II</td>
<td>1010</td>
</tr>
<tr>
<td>419.</td>
<td>Presynaptic mechanisms IV</td>
<td>1013</td>
</tr>
<tr>
<td>420.</td>
<td>Ion channels: ligand-gated I</td>
<td>1015</td>
</tr>
<tr>
<td>421.</td>
<td>Ion channels: ligand-gated II</td>
<td>1018</td>
</tr>
<tr>
<td>422.</td>
<td>Peptides: physiological effects III</td>
<td>1020</td>
</tr>
<tr>
<td>423.</td>
<td>Opioids: anatomy and physiology I</td>
<td>1023</td>
</tr>
<tr>
<td>424.</td>
<td>Opioids: anatomy and physiology II</td>
<td>1026</td>
</tr>
<tr>
<td>425.</td>
<td>Peptides: biosynthesis and metabolism II</td>
<td>1029</td>
</tr>
<tr>
<td>426.</td>
<td>Serotonin III</td>
<td>1031</td>
</tr>
<tr>
<td>427.</td>
<td>SHT\textsubscript{\textalpha} and other SHT receptor subtypes</td>
<td>1034</td>
</tr>
<tr>
<td>428.</td>
<td>Receptor modulation: up and down regulation I</td>
<td>1037</td>
</tr>
<tr>
<td>429.</td>
<td>GABA\textsubscript{\textalpha} receptors</td>
<td>1040</td>
</tr>
<tr>
<td>430.</td>
<td>Excitatory amino acids: NMDA receptor glycine and polyamine sites</td>
<td>1041</td>
</tr>
<tr>
<td>431.</td>
<td>Catecholamines IV</td>
<td>1044</td>
</tr>
<tr>
<td>432.</td>
<td>Catecholamines V</td>
<td>1047</td>
</tr>
<tr>
<td>433.</td>
<td>Behavioral pharmacology: dopamine and hormones I</td>
<td>1051</td>
</tr>
<tr>
<td>434.</td>
<td>Acetylcholine II</td>
<td>1055</td>
</tr>
<tr>
<td>435.</td>
<td>Acetylcholine—receptors: muscarinic III</td>
<td>1058</td>
</tr>
<tr>
<td>436.</td>
<td>Respiratory control</td>
<td>1061</td>
</tr>
<tr>
<td>437.</td>
<td>Regulation of autonomic function: control of lumbosacral autonomic outflow</td>
<td>1064</td>
</tr>
<tr>
<td>438.</td>
<td>Neuroendocrine regulation: oxytocin, vasopressin</td>
<td>1067</td>
</tr>
<tr>
<td>439.</td>
<td>Hypothalamic-pituitary-adrenal regulation: steroids</td>
<td>1070</td>
</tr>
<tr>
<td>440.</td>
<td>Pain—pathways: response to injury</td>
<td>1072</td>
</tr>
<tr>
<td>441.</td>
<td>Sensory systems—retina: retinal chemistry and anatomy</td>
<td>1074</td>
</tr>
<tr>
<td>442.</td>
<td>Sensory systems—auditory system: hair cells and cochlea II</td>
<td>1078</td>
</tr>
<tr>
<td>443.</td>
<td>Somatosensory cortex and thalamocortical relationships III</td>
<td>1080</td>
</tr>
<tr>
<td>444.</td>
<td>Motor systems and sensorimotor integration: oculomotor system III</td>
<td>1082</td>
</tr>
<tr>
<td>445.</td>
<td>Control of posture and movement: arm and hand</td>
<td>1085</td>
</tr>
</tbody>
</table>

### Session Number and Title

<table>
<thead>
<tr>
<th>Session</th>
<th>Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>446.</td>
<td>Circuity and pattern generation II</td>
<td>1090</td>
</tr>
<tr>
<td>447.</td>
<td>Association cortex and thalamocortical relationships</td>
<td>1093</td>
</tr>
<tr>
<td>448.</td>
<td>Hippocampus and amygdala: neurophysiology II</td>
<td>1096</td>
</tr>
<tr>
<td>449.</td>
<td>Neuroethology: avian song</td>
<td>1098</td>
</tr>
<tr>
<td>450.</td>
<td>Drugs of abuse: cannabinoids, nicotine and PCP</td>
<td>1100</td>
</tr>
<tr>
<td>451.</td>
<td>Drugs of abuse</td>
<td>1103</td>
</tr>
<tr>
<td>452.</td>
<td>Epilepsy: kindling I</td>
<td>1105</td>
</tr>
<tr>
<td>453.</td>
<td>Degenerative disease—Parkinson's: humans and treatment</td>
<td>1108</td>
</tr>
<tr>
<td>454.</td>
<td>Neurotoxicity: amino acids</td>
<td>1111</td>
</tr>
<tr>
<td>455.</td>
<td>Neurotoxicity: other I</td>
<td>1113</td>
</tr>
<tr>
<td>456.</td>
<td>Neurotoxicity: other II</td>
<td>1116</td>
</tr>
<tr>
<td>457.</td>
<td>Degenerative disease—other: basal ganglia</td>
<td>1119</td>
</tr>
</tbody>
</table>

### Presidential Special Lecture—11:45 a.m.

<table>
<thead>
<tr>
<th>Session</th>
<th>Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>458.</td>
<td>Target Regulation of Neuronal Phenotype</td>
<td>1121</td>
</tr>
<tr>
<td></td>
<td>S. Landis</td>
<td>No abstract</td>
</tr>
</tbody>
</table>

### Symposia—1:00 p.m.

<table>
<thead>
<tr>
<th>Session</th>
<th>Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>459.</td>
<td>Regulation of Nicotinic Acetylcholine Receptor Expression and Function</td>
<td>1124</td>
</tr>
<tr>
<td></td>
<td>Chaired by: R.J. Lukas</td>
<td>1124</td>
</tr>
<tr>
<td>460.</td>
<td>Neuronal Regulation of Renal Function: A Model System for Nervous System Interactions</td>
<td>1121</td>
</tr>
<tr>
<td></td>
<td>Chaired by: J.M. Wyss</td>
<td>1121</td>
</tr>
</tbody>
</table>

### Slide Sessions—1:00 p.m.

<table>
<thead>
<tr>
<th>Session</th>
<th>Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>461.</td>
<td>Excitotoxicity V</td>
<td>1124</td>
</tr>
<tr>
<td>462.</td>
<td>Molecular neurobiology of 5HT receptors</td>
<td>1123</td>
</tr>
<tr>
<td>463.</td>
<td>Process outgrowth, growth cones and guidance mechanisms X</td>
<td>1125</td>
</tr>
<tr>
<td>464.</td>
<td>Development and plasticity—visual system: connections</td>
<td>1128</td>
</tr>
<tr>
<td>465.</td>
<td>Circuity and pattern generation III</td>
<td>1130</td>
</tr>
<tr>
<td>466.</td>
<td>Cortex IV</td>
<td>1132</td>
</tr>
<tr>
<td>467.</td>
<td>Nerve growth factors IX</td>
<td>1134</td>
</tr>
<tr>
<td>468.</td>
<td>Receptor modulation: up and down regulation II</td>
<td>1136</td>
</tr>
<tr>
<td>469.</td>
<td>Genetic models of nervous disorders III</td>
<td>1138</td>
</tr>
<tr>
<td>470.</td>
<td>Localization of neurotransmitter receptor subtypes</td>
<td>1140</td>
</tr>
<tr>
<td>471.</td>
<td>Peptides—receptors, metabolism and actions</td>
<td>1141</td>
</tr>
<tr>
<td>472.</td>
<td>Pain: pathways II</td>
<td>1143</td>
</tr>
<tr>
<td>473.</td>
<td>Serotonin IV</td>
<td>1145</td>
</tr>
</tbody>
</table>

### Poster Sessions—1:00 p.m.

<table>
<thead>
<tr>
<th>Session</th>
<th>Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>474.</td>
<td>Differentiation, morphogenesis and development: neurogenesis and survival</td>
<td>1147</td>
</tr>
<tr>
<td>475.</td>
<td>Differentiation, morphogenesis and development: tissue culture models</td>
<td>1149</td>
</tr>
<tr>
<td>476.</td>
<td>Differentiation, morphogenesis and development: forebrain</td>
<td>1150</td>
</tr>
<tr>
<td>477.</td>
<td>Differentiation, morphogenesis and development: position and form</td>
<td>1153</td>
</tr>
<tr>
<td>478.</td>
<td>Transplantation: receptor expression</td>
<td>1154</td>
</tr>
<tr>
<td>479.</td>
<td>Regeneration: enhancement factors</td>
<td>1156</td>
</tr>
<tr>
<td>480.</td>
<td>The aging process: cell biology, morphometry, other</td>
<td>1158</td>
</tr>
<tr>
<td>481.</td>
<td>Neural plasticity in adult animals IV</td>
<td>1162</td>
</tr>
<tr>
<td>482.</td>
<td>Neuroglia and myelin IV</td>
<td>1165</td>
</tr>
<tr>
<td>483.</td>
<td>Gene structure and function IV</td>
<td>1168</td>
</tr>
<tr>
<td>484.</td>
<td>mRNA regulation: neuropeptides</td>
<td>1170</td>
</tr>
<tr>
<td>485.</td>
<td>Calcium regulation: neuropeptides</td>
<td>1172</td>
</tr>
<tr>
<td>486.</td>
<td>Calcium channels: molecular properties</td>
<td>1174</td>
</tr>
<tr>
<td>Session Number and Title</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>487. Catecholamines VI</td>
<td>1176</td>
<td></td>
</tr>
<tr>
<td>488. Second messengers V</td>
<td>1179</td>
<td></td>
</tr>
<tr>
<td>489. Excitatory amino acids: non-NMDA receptors</td>
<td>1181</td>
<td></td>
</tr>
<tr>
<td>490. Excitatory amino acids: anatomy and physiology I</td>
<td>1184</td>
<td></td>
</tr>
<tr>
<td>491. Excitatory amino acids: anatomy and physiology II</td>
<td>1187</td>
<td></td>
</tr>
<tr>
<td>492. Behavioral pharmacology: opiates, NMDA and others</td>
<td>1191</td>
<td></td>
</tr>
<tr>
<td>493. Cellular biology of 5HT receptors</td>
<td>1194</td>
<td></td>
</tr>
<tr>
<td>494. Neural-immune interactions: stress and behavior</td>
<td>1196</td>
<td></td>
</tr>
<tr>
<td>495. Hypothalamic-pituitary-gonadal regulation: modulation by steroids and monoamines</td>
<td>1200</td>
<td></td>
</tr>
<tr>
<td>496. Regulation of autonomic function: temperature regulation and neural-immune system interactions</td>
<td>1203</td>
<td></td>
</tr>
<tr>
<td>497. Neural-immune interactions I</td>
<td>1205</td>
<td></td>
</tr>
<tr>
<td>498. Neural-immune interactions II</td>
<td>1209</td>
<td></td>
</tr>
<tr>
<td>499. Neural-immune interactions: interleukins</td>
<td>1211</td>
<td></td>
</tr>
<tr>
<td>500. Somatosensory cortex and thalamocortical relationships IV</td>
<td>1214</td>
<td></td>
</tr>
<tr>
<td>501. Sensory systems—retina: retinal ganglion cells</td>
<td>1216</td>
<td></td>
</tr>
<tr>
<td>502. Sensory systems—visual cortex: response properties</td>
<td>1218</td>
<td></td>
</tr>
<tr>
<td>503. Cortex V</td>
<td>1221</td>
<td></td>
</tr>
<tr>
<td>504. Invertebrate motor function</td>
<td>1224</td>
<td></td>
</tr>
<tr>
<td>505. Basal ganglia and thalamus VII</td>
<td>1228</td>
<td></td>
</tr>
<tr>
<td>506. Basal ganglia and thalamus VIII</td>
<td>1231</td>
<td></td>
</tr>
<tr>
<td>507. Brainstem systems</td>
<td>1233</td>
<td></td>
</tr>
<tr>
<td>508. Learning and memory—pharmacology: monoamines</td>
<td>1236</td>
<td></td>
</tr>
<tr>
<td>509. Human behavioral neurobiology: history, memory, imaging, other</td>
<td>1239</td>
<td></td>
</tr>
<tr>
<td>510. Ingestive behavior: salt, water and aversion</td>
<td>1242</td>
<td></td>
</tr>
<tr>
<td>511. Learning and memory: spatial</td>
<td>1245</td>
<td></td>
</tr>
<tr>
<td>512. Neuroethology: avian song and other</td>
<td>1249</td>
<td></td>
</tr>
<tr>
<td>513. Ingestive behavior: body weight and eating</td>
<td>1251</td>
<td></td>
</tr>
<tr>
<td>514. Biological rhythms and sleep IV</td>
<td>1254</td>
<td></td>
</tr>
<tr>
<td>515. Neurotoxicity: MPTP</td>
<td>1257</td>
<td></td>
</tr>
<tr>
<td>516. Clinical CNS neurophysiology</td>
<td>1260</td>
<td></td>
</tr>
<tr>
<td>517. Alzheimer’s disease: neuropathology III</td>
<td>1263</td>
<td></td>
</tr>
<tr>
<td>518. Epilepsy: kindling II</td>
<td>1265</td>
<td></td>
</tr>
<tr>
<td>519. Alzheimer’s disease: molecular studies</td>
<td>1267</td>
<td></td>
</tr>
</tbody>
</table>

Special Lecture—4:15 p.m.

520. An Introductory Survey of Chaos
M. Feigenbaum .......................................................... No abstract

---

**FRIDAY**

**Symposia—8:30 a.m.**

521. Glia: Synthesis of and Responses to Growth Factors and Neuropeptides
Chair by: J.P. Schwartz ........................................... 1269

---

<table>
<thead>
<tr>
<th>Session Number and Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>522. Physiology of Peptidergic Nerve Terminals in the Vertebrate Neurohypophysis</td>
<td>1269</td>
</tr>
</tbody>
</table>

**Slide Sessions—8:30 a.m.**

523. Sensory systems—visual cortex: intracortical interactions ........................................... 1269
| 524. Cell lineage III | 1271 |
| 525. Calcium channels IV | 1273 |
| 526. mRNA regulation: peptides and c-fos | 1275 |
| 527. Ischemia VII | 1277 |
| 528. Somatic and visceral afferents IV | 1279 |

**Poster Sessions—8:30 a.m.**

529. Regeneration: chambers and grafts ..................................................... 1281
<p>| 530. Transplantation: expression of specific neuronal markers | 1283 |
| 531. Transplantation: general III | 1285 |
| 532. Development and plasticity—visual system: retinotectal connections | 1287 |
| 533. Synaptogenesis in the CNS II | 1290 |
| 534. Ontogeny of neuroendocrine systems | 1291 |
| 535. mRNA regulation: transmitter enzymes and receptors II | 1293 |
| 536. Ion channels: cell function | 1295 |
| 537. 5HT receptors | 1298 |
| 538. Second messengers VI | 1300 |
| 539. Receptor modulation: up and down regulation III | 1304 |
| 540. Acetylcholine III | 1306 |
| 541. Localization of peptide hormones and monoamines | 1309 |
| 542. Neuroendocrine regulation: other III | 1311 |
| 543. Sensory systems—subcortical visual pathways: midbrain, etc. | 1313 |
| 544. Control of posture and movement: clinical studies | 1315 |
| 545. Control of posture and movement: humans | 1318 |
| 546. Hippocampus and amygdala: behavior | 1321 |
| 547. Psychotherapeutic drugs | 1322 |
| 548. Neuroethology: fish | 1325 |
| 549. Learning and memory—pharmacology | 1329 |
| 550. Biological rhythms and sleep V | 1332 |
| 551. Epilepsy: animal models | 1335 |
| 552. Trauma: spinal cord, NMDA and other | 1338 |
| 553. Degenerative disease—Parkinson’s: MPTP monkeys and rodents | 1340 |
| 554. Degenerative disease—other: MS, ALS and others | 1343 |
| 555. Alzheimer’s disease: models | 1346 |
| 556. Mental illness: schizophrenia | 1348 |</p>
<table>
<thead>
<tr>
<th>Session Number</th>
<th>Session Title</th>
<th>Type</th>
<th>Mon.</th>
<th>Tue.</th>
<th>Wed.</th>
<th>Thu.</th>
<th>Fri.</th>
</tr>
</thead>
<tbody>
<tr>
<td>79.</td>
<td>Cell lineage I</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>273.</td>
<td>Cell lineage II</td>
<td>Poster</td>
<td></td>
<td>wAM</td>
<td></td>
<td>fAM</td>
<td></td>
</tr>
<tr>
<td>524.</td>
<td>Cell lineage III</td>
<td>Slide</td>
<td>tuAM</td>
<td></td>
<td>thAM</td>
<td>fAM</td>
<td></td>
</tr>
<tr>
<td>135.</td>
<td>Cellular and molecular studies I</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>334.</td>
<td>Cellular and molecular studies II</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>404.</td>
<td>Cellular and molecular studies III</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>464.</td>
<td>Development and plasticity—visual system: connections</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>213.</td>
<td>Development and plasticity—visual system: cortical anatomy</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>331.</td>
<td>Development and plasticity—visual system: molecular and cellular mechanisms I</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>409.</td>
<td>Development and plasticity—visual system: molecular and cellular mechanisms II</td>
<td>Poster</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>147.</td>
<td>Development and plasticity—visual system: retina and optic nerve</td>
<td>Poster</td>
<td></td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>532.</td>
<td>Development and plasticity—visual system: retinotectal connections</td>
<td>Poster</td>
<td>fAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>82.</td>
<td>Differentiation, morphogenesis and development: cell surface and matrix components</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>81.</td>
<td>Differentiation, morphogenesis and development: currents, channels, muscle development</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>83.</td>
<td>Differentiation, morphogenesis and development: cytoskeleton</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>141.</td>
<td>Differentiation, morphogenesis and development: fiber guidance and synaptogenesis</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>476.</td>
<td>Differentiation, morphogenesis and development: forebrain</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80.</td>
<td>Differentiation, morphogenesis and development: glia</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>271.</td>
<td>Differentiation, morphogenesis and development: molecular correlates</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>474.</td>
<td>Differentiation, morphogenesis and development: neurogenesis and survival</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>272.</td>
<td>Differentiation, morphogenesis and development: neurotransmitters, peptides, hormones</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>477.</td>
<td>Differentiation, morphogenesis and development: position and form</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>475.</td>
<td>Differentiation, morphogenesis and development: tissue culture models</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>143.</td>
<td>Endocrine control and development I</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>144.</td>
<td>Endocrine control and development II</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>196.</td>
<td>Formation and maturation of synaptic connections</td>
<td>Slide</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>391.</td>
<td>Genetically Modified Cells: Development and Applications for the Neurosciences</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>521.</td>
<td>Glia: Synthesis of and Responses to Growth Factors and Neuropeptides</td>
<td>Poster</td>
<td>fAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>351.</td>
<td>Limbic system II</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65.</td>
<td>Long-term potentiation I</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>212.</td>
<td>Long-term potentiation II</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>274.</td>
<td>Long-term potentiation III</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>348.</td>
<td>Long-term potentiation IV</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>407.</td>
<td>Long-term potentiation V</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Molecular Mechanisms of Neuronal Guidance</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>145.</td>
<td>Motor systems: development and plasticity I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>146.</td>
<td>Motor systems: development and plasticity II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70.</td>
<td>Nerve growth factors I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>132.</td>
<td>Nerve growth factors II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>207.</td>
<td>Nerve growth factors III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>208.</td>
<td>Nerve growth factors IV</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>342.</td>
<td>Nerve growth factors V</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>343.</td>
<td>Nerve growth factors VI</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>410.</td>
<td>Nerve growth factors VII</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>411.</td>
<td>Nerve growth factors VIII</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>467.</td>
<td>Nerve growth factors IX</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.</td>
<td>Neural plasticity in adult animals I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150.</td>
<td>Neural plasticity in adult animals II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session Number</td>
<td>Session Title</td>
<td>Type</td>
<td>Mon.</td>
<td>Tue.</td>
<td>Wed.</td>
<td>Thu.</td>
<td>Fri.</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>276.</td>
<td>Neural plasticity in adult animals III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>481.</td>
<td>Neural plasticity in adult animals IV</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>347.</td>
<td>Neuronal death: deafferentation and prenatal studies</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>408.</td>
<td>Neuronal death: lesion studies</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>78.</td>
<td>Neuronal death: mechanisms</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>456.</td>
<td>Neurotoxicity: other II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>350.</td>
<td>Neurotransmitter and neuromodulator development</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.</td>
<td>Nutritional and prenatal factors</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>149.</td>
<td>Ontogeny of dopaminergic systems</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>534.</td>
<td>Ontogeny of neuroendocrine systems</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>209.</td>
<td>Other trophic agents I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>413.</td>
<td>Other trophic agents II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>68.</td>
<td>Process outgrowth, growth cones and guidance mechanisms I</td>
<td>Slide</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>139.</td>
<td>Process outgrowth, growth cones and guidance mechanisms II</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>140.</td>
<td>Process outgrowth, growth cones and guidance mechanisms III</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>197.</td>
<td>Process outgrowth, growth cones and guidance mechanisms IV</td>
<td>Slide</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>262.</td>
<td>Process outgrowth, growth cones and guidance mechanisms V</td>
<td>Slide</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>338.</td>
<td>Process outgrowth, growth cones and guidance mechanisms VI</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>339.</td>
<td>Process outgrowth, growth cones and guidance mechanisms VII</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>416.</td>
<td>Process outgrowth, growth cones and guidance mechanisms VIII</td>
<td>Poster</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>417.</td>
<td>Process outgrowth, growth cones and guidance mechanisms IX</td>
<td>Poster</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>463.</td>
<td>Process outgrowth, growth cones and guidance mechanisms X</td>
<td>Slide</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>529.</td>
<td>Regeneration: chambers and grafts</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>479.</td>
<td>Regeneration: enhancement factors</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>fAM</td>
</tr>
<tr>
<td>414.</td>
<td>Regeneration: general</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thAM</td>
</tr>
<tr>
<td>74.</td>
<td>Regeneration: general studies and molecular correlates</td>
<td>Slide</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>77.</td>
<td>Regeneration: genes, inhibitory factors and axonal transport</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>148.</td>
<td>Regeneration: molecular correlates</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>211.</td>
<td>Regeneration: specificity and functional recovery</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>265.</td>
<td>Sensory systems—development and plasticity I</td>
<td>Slide</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>344.</td>
<td>Sensory systems—development and plasticity II</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>345.</td>
<td>Sensory systems—development and plasticity III</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>336.</td>
<td>Specificity and enhancement of regeneration</td>
<td>Slide</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>142.</td>
<td>Specificity of synaptic connections</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>340.</td>
<td>Sprouting and sprouting mechanisms</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>wPM</td>
</tr>
<tr>
<td>20.</td>
<td>Substances of abuse</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24.</td>
<td>Synaptogenesis in the CNS I</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>533.</td>
<td>Synaptogenesis in the CNS II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>fAM</td>
</tr>
<tr>
<td>415.</td>
<td>Synaptogenesis of neuromuscular junctions</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thAM</td>
</tr>
<tr>
<td>480.</td>
<td>The aging process: cell biology, morphometry, other</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thPM</td>
</tr>
<tr>
<td>349.</td>
<td>The aging process: learning and memory, growth factors, physiology</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>wPM</td>
</tr>
<tr>
<td>19.</td>
<td>The aging process: neurotransmitters and endocrine regulation</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>210.</td>
<td>Transplantation: anatomical projections</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>275.</td>
<td>Transplantation: behavioral and electrophysiological effects</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>530.</td>
<td>Transplantation: expression of specific neuronal markers</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>fAM</td>
</tr>
<tr>
<td>22.</td>
<td>Transplantation: general I</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>203.</td>
<td>Transplantation: general II</td>
<td>Slide</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>531.</td>
<td>Transplantation: general III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>fAM</td>
</tr>
<tr>
<td>23.</td>
<td>Transplantation: new techniques and cell lines</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>399.</td>
<td>Transplantation: new techniques, immune rejection and behavior</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thAM</td>
</tr>
<tr>
<td>478.</td>
<td>Transplantation: receptor expression</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thPM</td>
</tr>
<tr>
<td>346.</td>
<td>Transplantation: transmitter expression</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>wPM</td>
</tr>
<tr>
<td>341.</td>
<td>Trophic interactions I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thAM</td>
</tr>
<tr>
<td>412.</td>
<td>Trophic interactions II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>thAM</td>
</tr>
</tbody>
</table>

**Theme B: Cell Biology**

<p>| 26.          | Blood-brain barrier                                                         | Poster | mAM  |      |      |      |      |
| 27.          | Cytoskeleton, transport and membrane targeting                              | Poster | mAM  |      |      |      |      |
| 71.          | Gene structure and function I                                               | Slide  | mPM  |      |      |      |      |
| 154.         | Gene structure and function II                                              | Poster | tuAM |      |      |      |      |
| 278.         | Gene structure and function III                                             | Poster |      |      |      |      | wAM  |
| 483.         | Gene structure and function IV                                              | Poster |      |      |      |      | thPM |
| 214.         | Membrane composition and cell surface macromolecules I                     | Poster | tuPM |      |      |      |      |</p>
<table>
<thead>
<tr>
<th>Session Number</th>
<th>Session Title</th>
<th>Type</th>
<th>Mon.</th>
<th>Tue.</th>
<th>Wed.</th>
<th>Thu.</th>
<th>Fri.</th>
</tr>
</thead>
<tbody>
<tr>
<td>418</td>
<td>Membrane composition and cell surface macromolecules II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>277</td>
<td>mRNA regulation: endocrine connection</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>151</td>
<td>mRNA regulation: general</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>484</td>
<td>mRNA regulation: neuropeptides</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>526</td>
<td>mRNA regulation: peptides and c-fos</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>352</td>
<td>mRNA regulation: transcription factors</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>397</td>
<td>mRNA regulation: transmitter enzymes and receptors I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>535</td>
<td>mRNA regulation: transmitter enzymes and receptors II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>153</td>
<td>Neuroglia and myelin I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>279</td>
<td>Neuroglia and myelin II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>402</td>
<td>Neuroglia and myelin III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>482</td>
<td>Neuroglia and myelin IV</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Staining, tracing and imaging techniques I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Staining, tracing and imaging techniques II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>152</td>
<td>Staining, tracing and imaging techniques III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Theme C: Excitable Membranes and Synaptic Transmission</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>261</td>
<td>Calcium channels I</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>283</td>
<td>Calcium channels II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>395</td>
<td>Calcium channels III</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>525</td>
<td>Calcium channels IV</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>485</td>
<td>Calcium channels and cellular calcium</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>486</td>
<td>Calcium channels: molecular properties</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>219</td>
<td>Calcium channels: pharmacology</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>125. Direct Ion Channel Gating by Intracellular Ions and Molecules</strong></td>
<td>Symp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>536</td>
<td>Ion channels: cell function</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>280</td>
<td>Ion channels: chloride and other channels</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>420</td>
<td>Ion channels: ligand-gated I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>421</td>
<td>Ion channels: ligand-gated II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>Ion channels: modulation and regulation I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>155</td>
<td>Ion channels: modulation and regulation II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>329</td>
<td>Ion channels: modulation and regulation III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>522. Physiology of Peptidergic Nerve Terminals in the</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vertebrate Neurohypophysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Postsynaptic mechanisms I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>202</td>
<td>Postsynaptic mechanisms II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Potassium channels I</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>156</td>
<td>Potassium channels II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>218</td>
<td>Potassium channels III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>281</td>
<td>Potassium channels IV</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>76</td>
<td>Presynaptic mechanisms I</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>216</td>
<td>Presynaptic mechanisms II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>217</td>
<td>Presynaptic mechanisms III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>419</td>
<td>Presynaptic mechanisms IV</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>84</td>
<td>Sodium channels I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>85</td>
<td>Sodium channels II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>215</td>
<td>Synaptic structure and function I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>282</td>
<td>Synaptic structure and function II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Synaptic transmission</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Theme D: Neurotransmitters, Modulators, and Receptors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>353</td>
<td>SHT receptors: behavior and pharmacology</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>427</td>
<td>SHT₁a and other SHT receptor subtypes</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>537</td>
<td>SHT, receptors</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>Acetylcholine I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>434</td>
<td>Acetylcholine II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>540</td>
<td>Acetylcholine III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Acetylcholine—receptors</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>92</td>
<td>Acetylcholine—receptors: muscarinic I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>227</td>
<td>Acetylcholine—receptors: muscarinic II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>435</td>
<td>Acetylcholine—receptors: muscarinic III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>93</td>
<td>Acetylcholine—receptors: nicotinic I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>285</td>
<td>Acetylcholine—receptors: nicotinic II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session Number</td>
<td>Session Title</td>
<td>Type</td>
<td>Day &amp; Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>--------</td>
<td>------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>165</td>
<td>Behavioral pharmacology: acetylcholine, monoamines, and benzodiazepines</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>433</td>
<td>Behavioral pharmacology: dopamine and hormones</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>492</td>
<td>Behavioral pharmacology: opiates, NMDA and others</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>Brain glutamate systems</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Catecholamines I</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>224</td>
<td>Catecholamines II</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>332</td>
<td>Catecholamines III</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>431</td>
<td>Catecholamines IV</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>432</td>
<td>Catecholamines V</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>487</td>
<td>Catecholamines VI</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>164</td>
<td>Cell biology of catecholamine receptors</td>
<td>Slide</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Cellular and molecular biology of catecholamine receptors</td>
<td>Slide</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>493</td>
<td>Cellular biology of 5HT receptors</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Dopamine physiology I</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>226</td>
<td>Dopamine physiology II</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>490</td>
<td>Excitatory amino acids: anatomy and physiology I</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>491</td>
<td>Excitatory amino acids: anatomy and physiology II</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>356</td>
<td>Excitatory amino acids: NMDA receptor antagonians</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>430</td>
<td>Excitatory amino acids: NMDA receptor glycine and polyamine sites</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>489</td>
<td>Excitatory amino acids: non-NMDA receptors</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>161</td>
<td>Excitatory amino acids: pharmacology I</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>Excitatory amino acids: pharmacology II</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>284</td>
<td>Excitatory amino acids: pharmacology III</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>Excitatory amino acids: receptors I</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Excitatory amino acids: receptors II</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>229</td>
<td>Excitatory amino acids: receptors III</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>230</td>
<td>Excitatory amino acids: receptors IV</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>231</td>
<td>Excitatory amino acids: receptors V</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>259</td>
<td>Excitatory amino acids: receptors VI</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>396</td>
<td>Excitatory amino acids: receptors VII</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>88</td>
<td>Excitotoxity I</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>89</td>
<td>Excitotoxity II</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>Excitotoxity III</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>128</td>
<td>Excitotoxity IV</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>461</td>
<td>Excitotoxity V</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>GABA_A receptors I</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>162</td>
<td>GABA_A receptors II</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>289</td>
<td>GABA_A receptors III</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>335</td>
<td>GABA_A receptors IV</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>429</td>
<td>GABA_A receptors</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>257</td>
<td>Galanin: Multidisciplinary Studies</td>
<td>Symp.</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>134</td>
<td>Interactions between neurotransmitters I</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>223</td>
<td>Interactions between neurotransmitters II</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>354</td>
<td>Interactions between neurotransmitters III</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>290</td>
<td>Localization of 5HT receptor subtypes</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>470</td>
<td>Localization of neurotransmitter receptor subtypes</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>288</td>
<td>Localization of neurotransmitter receptors I</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>291</td>
<td>Localization of neurotransmitter receptors II</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>541</td>
<td>Localization of peptide hormones and monoamines</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>287</td>
<td>Mechanisms in transporter physiology</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94</td>
<td>Molecular biology of dopamine receptors</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>462</td>
<td>Molecular neurobiology of 5HT receptors</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Neurochemistry of transmitter systems</td>
<td>Poster</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>423</td>
<td>Opioids: anatomy and physiology I</td>
<td>Poster</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>424</td>
<td>Opioids: anatomy and physiology II</td>
<td>Poster</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>Opioids: behavior</td>
<td>Poster</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>158</td>
<td>Opioids: receptors I</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>159</td>
<td>Opioids: receptors II</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>333</td>
<td>Opioids: receptors III</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>Other biogenic amines and purines</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>157</td>
<td>Peptides: anatomical localization I</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>222</td>
<td>Peptides: anatomical localization II</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session Number</td>
<td>Session Title</td>
<td>Type</td>
<td>Mon.</td>
<td>Tue.</td>
<td>Wed.</td>
<td>Thu.</td>
<td>Fri.</td>
</tr>
<tr>
<td>----------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>166.</td>
<td>Peptides: biosynthesis and metabolism I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>425.</td>
<td>Peptides: biosynthesis and metabolism II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>328.</td>
<td>Peptides: biosynthesis, metabolism and biochemical characterization</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>87.</td>
<td>Peptides: physiological effects I</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>221.</td>
<td>Peptides: physiological effects II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>422.</td>
<td>Peptides: physiological effects III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Peptides—receptors</td>
<td>Slide</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>471.</td>
<td>Peptides—receptors, metabolism and actions</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>286.</td>
<td>Peptides—receptors: angiotensin, endothelin</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38.</td>
<td>Peptides—receptors: CCK, somatostatin</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>220.</td>
<td>Peptides—receptors: other</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>428.</td>
<td>Receptor modulation: up and down regulation I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>468.</td>
<td>Receptor modulation: up and down regulation II</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>539.</td>
<td>Receptor modulation: up and down regulation III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>163.</td>
<td>Regulation of catecholamine receptors</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>459.</td>
<td>Regulation of Nicotinic Acetylcholine Receptor Expression and Function</td>
<td>Symp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>160.</td>
<td>Second messengers I</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>228.</td>
<td>Second messengers II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>264.</td>
<td>Second messengers III</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400.</td>
<td>Second messengers IV</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>488.</td>
<td>Second messengers V</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>538.</td>
<td>Second messengers VI</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35.</td>
<td>Serotonin I</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>225.</td>
<td>Serotonin II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>426.</td>
<td>Serotonin III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>473.</td>
<td>Serotonin IV</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>137.</td>
<td>Transmitters in invertebrates I</td>
<td>Slide</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>232.</td>
<td>Transmitters in invertebrates II</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>266.</td>
<td>Transmitters in invertebrates III</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>355.</td>
<td>Transmitters in invertebrates IV</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Theme E: Endocrine and Autonomic Regulation**

<table>
<thead>
<tr>
<th>Session Number</th>
<th>Session Title</th>
<th>Type</th>
<th>Mon.</th>
<th>Tue.</th>
<th>Wed.</th>
<th>Thu.</th>
<th>Fri.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.</td>
<td>Cardiovascular regulation I</td>
<td>Slide</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>133.</td>
<td>Cardiovascular regulation II</td>
<td>Slide</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>98.</td>
<td>Cardiovascular regulation: brainstem and baroreflexes</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>233.</td>
<td>Cardiovascular regulation: brainstem mechanisms</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>97.</td>
<td>Cardiovascular regulation: bulbospinal mechanisms</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>234.</td>
<td>Cardiovascular regulation: hypothalamic, limbic, and cortical mechanisms</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>195.</td>
<td>Hypothalamic-pituitary-adrenal regulation</td>
<td>Slide</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>41.</td>
<td>Hypothalamic-pituitary-adrenal regulation: CRF</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>292.</td>
<td>Hypothalamic-pituitary-adrenal regulation: other</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>439.</td>
<td>Hypothalamic-pituitary-adrenal regulation: steroids</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>126.</td>
<td>Hypothalamic-pituitary-gonadal regulation I</td>
<td>Slide</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>393.</td>
<td>Hypothalamic-pituitary-gonadal regulation II</td>
<td>Slide</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>168.</td>
<td>Hypothalamic-pituitary-gonadal regulation: GnRH</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>167.</td>
<td>Hypothalamic-pituitary-gonadal regulation: modulation by peptides</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>495.</td>
<td>Hypothalamic-pituitary-gonadal regulation: modulation by steroids and monoamines</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**460. Neuronal Regulation of Renal Function: A Model System for Nervous System Interactions**

<table>
<thead>
<tr>
<th>Session Number</th>
<th>Session Title</th>
<th>Type</th>
<th>Mon.</th>
<th>Tue.</th>
<th>Wed.</th>
<th>Thu.</th>
<th>Fri.</th>
</tr>
</thead>
<tbody>
<tr>
<td>269.</td>
<td>Regulation of autonomic and respiratory functions</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>437.</td>
<td>Regulation of autonomic function: control of lumbar sacral autonomic outflow</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session Number</td>
<td>Session Title</td>
<td>Type</td>
<td>Mon.</td>
<td>Tue.</td>
<td>Wed.</td>
<td>Thu.</td>
<td>Fri.</td>
</tr>
<tr>
<td>----------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>--------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>358.</td>
<td>Regulation of autonomic function: gastrointestinal control</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>357.</td>
<td>Regulation of autonomic function: peripheral autonomic organization and functions</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>496.</td>
<td>Regulation of autonomic function: temperature regulation and neural-immune system interactions</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>436.</td>
<td>Respiratory control</td>
<td>Poster</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>405.</td>
<td>Steroids: receptors and actions</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Theme F: Sensory Systems</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>192.</td>
<td>Brain Modulation of Sensory Signals</td>
<td>Symp.</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45.</td>
<td>Chemical senses: central pathways I</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>170.</td>
<td>Chemical senses: central pathways II</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>Chemical senses: peripheral mechanisms I</td>
<td>Slide</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>363.</td>
<td>Chemical senses: peripheral mechanisms II</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>364.</td>
<td>Chemical senses: peripheral mechanisms III</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>392.</td>
<td>Differential Processing of Visceral and Somatic Input in the Central Nervous System</td>
<td>Symp.</td>
<td></td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62.</td>
<td>Hair Cells of the Inner Ear: Structure, Transduction, and Active Motion</td>
<td>Symp.</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>169.</td>
<td>Invertebrate sensory systems</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43.</td>
<td>Pain modulation: anatomy and physiology I</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>44.</td>
<td>Pain modulation: anatomy and physiology II</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>73.</td>
<td>Pain modulation: anatomy and physiology III</td>
<td>Slide</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>236.</td>
<td>Pain modulation: anatomy and physiology IV</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>173.</td>
<td>Pain modulation: pharmacology I</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>237.</td>
<td>Pain modulation: pharmacology II</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>267.</td>
<td>Pain modulation: pharmacology III</td>
<td>Slide</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>172.</td>
<td>Pain modulation: spinal opioid pharmacology</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>294.</td>
<td>Pain: pathways I</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>472.</td>
<td>Pain: pathways II</td>
<td>Slide</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>440.</td>
<td>Pain—pathways: response to injury</td>
<td>Poster</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>298.</td>
<td>Sensory systems—auditory system: central pathways I</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>309.</td>
<td>Sensory systems—auditory system: central pathways II</td>
<td>Slide</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>299.</td>
<td>Sensory systems—auditory system: central physiology I</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>362.</td>
<td>Sensory systems—auditory system: central physiology II</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>361.</td>
<td>Sensory systems—auditory system: hair cells and cochlea I</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>442.</td>
<td>Sensory systems—auditory system: hair cells and cochlea II</td>
<td>Poster</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>360.</td>
<td>Sensory systems—auditory system: models</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300.</td>
<td>Sensory systems—auditory system structure: function of identified cells</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>171.</td>
<td>Sensory systems—retina: receptors, outer retina, ERG</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>441.</td>
<td>Sensory systems—retina: retinal chemistry and anatomy</td>
<td>Poster</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>201.</td>
<td>Sensory systems—retina: retinal circuits</td>
<td>Slide</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>501.</td>
<td>Sensory systems—retina: retinal ganglion cells</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>297.</td>
<td>Sensory systems—retina: retinal signals</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72.</td>
<td>Sensory systems—subcortical visual pathways: LGN</td>
<td>Slide</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>543.</td>
<td>Sensory systems—subcortical visual pathways: midbrain, etc.</td>
<td>Poster</td>
<td>fAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>296.</td>
<td>Sensory systems—subcortical visual pathways: retinal projections and thalamus</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47.</td>
<td>Sensory systems—subcortical visual pathways: superior colliculus and related</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>130.</td>
<td>Sensory systems—visual cortex: architecture and function</td>
<td>Slide</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>295.</td>
<td>Sensory systems—visual cortex: connections</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>238.</td>
<td>Sensory systems—visual cortex: evoked potentials and stimulation</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>260.</td>
<td>Sensory systems—visual cortex: extrastriate cortex</td>
<td>Slide</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>523.</td>
<td>Sensory systems—visual cortex: intracortical interactions</td>
<td>Slide</td>
<td>fAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Sensory systems—visual cortex: motion pathways</td>
<td>Slide</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>502.</td>
<td>Sensory systems—visual cortex: response properties</td>
<td>Poster</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>102.</td>
<td>Sensory systems—visual cortex: theoretical approaches</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46.</td>
<td>Sensory systems—visual psychophysics and behavior I</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>398.</td>
<td>Sensory systems—visual psychophysics and behavior II</td>
<td>Slide</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>175.</td>
<td>Somatic and visceral afferents I</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>293.</td>
<td>Somatic and visceral afferents II</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>365.</td>
<td>Somatic and visceral afferents III</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session Number</td>
<td>Session Title</td>
<td>Type</td>
<td>Mon.</td>
<td>Tue.</td>
<td>Wed.</td>
<td>Thu.</td>
<td>Fri.</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>528.</td>
<td>Somatic and visceral afferents IV</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>103.</td>
<td>Somatic and visceral afferents: capsaicin</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100.</td>
<td>Somatosensory cortex and thalamocortical relationships I</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>101.</td>
<td>Somatosensory cortex and thalamocortical relationships II</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>443.</td>
<td>Somatosensory cortex and thalamocortical relationships III</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500.</td>
<td>Somatosensory cortex and thalamocortical relationships IV</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>235.</td>
<td>Spinal cord: anatomy and physiology</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>174.</td>
<td>Spinal cord: neurotransmitters</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>99.</td>
<td>Subcortical somatosensory pathways</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Theme G: Motor Systems and Sensorimotor Integration**

<table>
<thead>
<tr>
<th>Session Number</th>
<th>Session Title</th>
<th>Type</th>
<th>Mon.</th>
<th>Tue.</th>
<th>Wed.</th>
<th>Thu.</th>
<th>Fri.</th>
</tr>
</thead>
<tbody>
<tr>
<td>104.</td>
<td>Basal ganglia and thalamus I</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>105.</td>
<td>Basal ganglia and thalamus II</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>176.</td>
<td>Basal ganglia and thalamus III</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>179.</td>
<td>Basal ganglia and thalamus IV</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>180.</td>
<td>Basal ganglia and thalamus V</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>394.</td>
<td>Basal ganglia and thalamus VI</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>505.</td>
<td>Basal ganglia and thalamus VII</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>506.</td>
<td>Basal ganglia and thalamus VIII</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>301.</td>
<td>Circuity and pattern generation I</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>446.</td>
<td>Circuity and pattern generation II</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>465.</td>
<td>Circuity and pattern generation III</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50.</td>
<td>Control of posture and movement I</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69.</td>
<td>Control of posture and movement II</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>368.</td>
<td>Control of posture and movement: animal locomotion</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>445.</td>
<td>Control of posture and movement: arm and hand</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>544.</td>
<td>Control of posture and movement: clinical studies</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>545.</td>
<td>Control of posture and movement: humans</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>369.</td>
<td>Control of posture and movement: learning and development</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>106.</td>
<td>Cortex I</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>107.</td>
<td>Cortex II</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>178.</td>
<td>Cortex III</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>466.</td>
<td>Cortex IV</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>503.</td>
<td>Cortex V</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>504.</td>
<td>Invertebrate motor function</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>268.</td>
<td>Motor systems and sensorimotor integration: cerebellum I</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>370.</td>
<td>Motor systems and sensorimotor integration: cerebellum II</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>371.</td>
<td>Motor systems and sensorimotor integration: cerebellum III</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>372.</td>
<td>Motor systems and sensorimotor integration: oculomotor system I</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>373.</td>
<td>Motor systems and sensorimotor integration: oculomotor system II</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>444.</td>
<td>Motor systems and sensorimotor integration: oculomotor system III</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>304.</td>
<td>Motor systems and sensorimotor integration: vestibular system I</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>305.</td>
<td>Motor systems and sensorimotor integration: vestibular system II</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>401.</td>
<td>Motor systems and sensorimotor integration: vestibular system III</td>
<td>Slide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52.</td>
<td>Muscle: general</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>177.</td>
<td>Muscle: human studies</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>51.</td>
<td>Muscle: molecular studies</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>367.</td>
<td>Reflex function: animal studies</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>366.</td>
<td>Reflex function: human</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**4. Regulation of Pattern Generating Networks**

<table>
<thead>
<tr>
<th>Session Number</th>
<th>Session Title</th>
<th>Type</th>
<th>Mon.</th>
<th>Tue.</th>
<th>Wed.</th>
<th>Thu.</th>
<th>Fri.</th>
</tr>
</thead>
<tbody>
<tr>
<td>48.</td>
<td>Spinal cord and brainstem</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>303.</td>
<td>Spinal cord and brainstem: anatomy</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>49.</td>
<td>Spinal cord and brainstem: motoneurons</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>302.</td>
<td>Spinal cord and brainstem: pharmacological studies</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Theme H: Other Systems of the CNS**

<table>
<thead>
<tr>
<th>Session Number</th>
<th>Session Title</th>
<th>Type</th>
<th>Mon.</th>
<th>Tue.</th>
<th>Wed.</th>
<th>Thu.</th>
<th>Fri.</th>
</tr>
</thead>
<tbody>
<tr>
<td>447.</td>
<td>Association cortex and thalamocortical relationships</td>
<td>Slide</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>129.</td>
<td>Brain metabolism and blood flow: central influences</td>
<td>Slide</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>239.</td>
<td>Brain metabolism and blood flow: endogenous factors</td>
<td>Slide</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>307.</td>
<td>Brain metabolism and blood flow: exogenous factors</td>
<td>Slide</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>Brain metabolism and blood flow: methods</td>
<td>Slide</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>507.</td>
<td>Brainstem systems</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>55.</td>
<td>Comparative neuroanatomy: fish and amphibia</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session Number</td>
<td>Session Title</td>
<td>Type</td>
<td>Mon.</td>
<td>Tue.</td>
<td>Wed.</td>
<td>Thu.</td>
<td>Fri.</td>
</tr>
<tr>
<td>----------------</td>
<td>------------------------------------------------------------------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>108</td>
<td>Comparative neuroanatomy: reptiles, birds, mammals</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>546</td>
<td>Hippocampus and amygdala: behavior</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>Hippocampus and amygdala: neuroanatomy</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>181</td>
<td>Hippocampus and amygdala: neurocytology</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>306</td>
<td>Hippocampus and amygdala: neurophysiology I</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>448</td>
<td>Hippocampus and amygdala: neurophysiology II</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>Hypothalamus</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>Limbic system I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Theme I: Neural Basis of Behavior**

248. Biological rhythms and sleep I

270. Biological rhythms and sleep II

317. Biological rhythms and sleep III

514. Biological rhythms and sleep IV

550. Biological rhythms and sleep V

451. Drugs of abuse

57. Drugs of abuse: alcohol I

112. Drugs of abuse: alcohol II

183. Drugs of abuse: alcohol III

198. Drugs of abuse: alcohol IV

312. Drugs of abuse: alcohol V

243. Drugs of abuse: amphetamine

136. Drugs of abuse: amphetamine and cocaine

450. Drugs of abuse: cannabinoids, nicotine and PCP

11. Drugs of abuse: cocaine

242. Drugs of abuse: cocaine and others

310. Drugs of abuse: cocaine, behavior

309. Drugs of abuse: cocaine, cellular

110. Drugs of abuse: cocaine, dopamine

111. Drugs of abuse: cocaine, serotonin

382. Drugs of abuse: opioids

204. Hormonal control of behavior I

308. Hormonal control of behavior II

315. Hormonal control of behavior III

380. Hormonal control of behavior IV

18. Human behavioral neurobiology

241. Human behavioral neurobiology: event related potentials, attention, audition

509. Human behavioral neurobiology: history, memory, imaging, other

513. Ingestive behavior: body weight and eating

376. Ingestive behavior: monoamines

131. Ingestive behavior: monoamines and nutrients

318. Ingestive behavior: peptides I

406. Ingestive behavior: peptides II

510. Ingestive behavior: salt, water and aversion

381. Interhemispheric relations

14. Invertebrate learning and behavior I

246. Invertebrate learning and behavior II

263. Invertebrate learning and behavior III

249. Learning and memory: anatomy I

250. Learning and memory: anatomy II

115. Learning and memory: conditioning

127. Learning and memory: human lesion studies

258. Learning and memory: non-human primate lesion studies

307. Learning and memory: pharmacology

58. Learning and memory—pharmacology: acetylcholine I

374. Learning and memory—pharmacology: acetylcholine II

316. Learning and memory—pharmacology: excitatory amino acids

508. Learning and memory—pharmacology: monoamines

113. Learning and memory: physiology I

185. Learning and memory: physiology II

205. Learning and memory: physiology III

314. Learning and memory: physiology IV

xix
<table>
<thead>
<tr>
<th>Session Number</th>
<th>Session Title</th>
<th>Type</th>
<th>Mon.</th>
<th>Tue.</th>
<th>Wed.</th>
<th>Thu.</th>
<th>Fri.</th>
</tr>
</thead>
<tbody>
<tr>
<td>378</td>
<td>Learning and memory: physiology V</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>511</td>
<td>Learning and memory: spatial</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>Monoamines and behavior I</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>Monoamines and behavior II</td>
<td>Slide</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>184</td>
<td>Monoamines and behavior III</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>311</td>
<td>Monoamines and behavior IV</td>
<td>Poster</td>
<td>tuPM</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>247</td>
<td>Motivation and emotion</td>
<td>Poster</td>
<td>tuPM</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>245</td>
<td>Motivation and self-stimulation</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>449</td>
<td>Neuroethology: avian song</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>512</td>
<td>Neuroethology: avian song and other</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>548</td>
<td>Neuroethology: fish</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>313</td>
<td>Neuroethology: invertebrates</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>379</td>
<td>Neuroethology: mammals, reptiles, amphibians</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>114</td>
<td>Neuropeptides and behavior I</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>186</td>
<td>Neuropeptides and behavior II</td>
<td>Poster</td>
<td>tuAM</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>375</td>
<td>Neuropeptides and behavior III</td>
<td>Poster</td>
<td>tuAM</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>547</td>
<td>Psychotherapeutic drugs</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>377</td>
<td>Psychotherapeutic drugs: antidepressants</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>109</td>
<td>Psychotherapeutic drugs: antipsychotics I</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>244</td>
<td>Psychotherapeutic drugs: antipsychotics II</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>Stress, hormones and the autonomic nervous system I</td>
<td>Slide</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>116</td>
<td>Stress, hormones and the autonomic nervous system II</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>182</td>
<td>Stress, hormones and the autonomic nervous system III</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>124</td>
<td>The Olivocerebellar System: Its Possible Role in Learning</td>
<td>Symp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Theme J: Disorders of the Nervous System**

<table>
<thead>
<tr>
<th>Session Number</th>
<th>Session Title</th>
<th>Type</th>
<th>Mon.</th>
<th>Tue.</th>
<th>Wed.</th>
<th>Thu.</th>
<th>Fri.</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Alzheimer’s disease: amyloid I</td>
<td>Slide</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>190</td>
<td>Alzheimer’s disease: amyloid II</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>322</td>
<td>Alzheimer’s disease: amyloid III</td>
<td>Poster</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Alzheimer’s disease: biochemistry and clinical studies</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>Alzheimer’s disease: cognitive and clinical studies</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>387</td>
<td>Alzheimer’s disease: cytoskeleton</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>555</td>
<td>Alzheimer’s disease: models</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>519</td>
<td>Alzheimer’s disease: molecular studies</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>199</td>
<td>Alzheimer’s disease: neuropathology I</td>
<td>Slide</td>
<td>tuPM</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>389</td>
<td>Alzheimer’s disease: neuropathology II</td>
<td>Poster</td>
<td>tuPM</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>517</td>
<td>Alzheimer’s disease: neuropathology III</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>252</td>
<td>Alzheimer’s disease: pharmacology</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>516</td>
<td>Clinical CNS neurophysiology</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>457</td>
<td>Degenerative disease—other: basal ganglia</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>554</td>
<td>Degenerative disease—other: MS, ALS and others</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>337</td>
<td>Degenerative disease—Parkinson’s</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>453</td>
<td>Degenerative disease—Parkinson’s: humans and treatment</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>553</td>
<td>Degenerative disease—Parkinson’s: MPTP monkeys and rodents</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>323</td>
<td>Disorders of the nervous system</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>320</td>
<td>Epilepsy: animal genetic models</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>551</td>
<td>Epilepsy: animal models</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>321</td>
<td>Epilepsy: anti-convulsant drugs</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Epilepsy: basic mechanisms I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>388</td>
<td>Epilepsy: basic mechanisms II</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>188</td>
<td>Epilepsy: human studies</td>
<td>Poster</td>
<td>tuAM</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>138</td>
<td>Epilepsy: human studies and animal models</td>
<td>Poster</td>
<td>tuAM</td>
<td>thAM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>452</td>
<td>Epilepsy: kindling I</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>518</td>
<td>Epilepsy: kindling II</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>119</td>
<td>Epilepsy: status epilepticus</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>383</td>
<td>Genetic models of nervous disorders I</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>469</td>
<td>Genetic models of nervous disorders III</td>
<td>Slide</td>
<td>tuPM</td>
<td>thPM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>253</td>
<td>Infectious diseases</td>
<td>Poster</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Ischemia I</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>117</td>
<td>Ischemia II</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>118</td>
<td>Ischemia III</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>384</td>
<td>Ischemia IV</td>
<td>Poster</td>
<td>mPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

XX
<table>
<thead>
<tr>
<th>Session Number</th>
<th>Session Title</th>
<th>Type</th>
<th>Mon.</th>
<th>Tue.</th>
<th>Wed.</th>
<th>Thu.</th>
<th>Fri.</th>
</tr>
</thead>
<tbody>
<tr>
<td>385.</td>
<td>Ischemia V</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>386.</td>
<td>Ischemia VI</td>
<td>Poster</td>
<td>wPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>527.</td>
<td>Ischemia VII</td>
<td>Slide</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td>fAM</td>
</tr>
<tr>
<td>59.</td>
<td>Mental illness: depression, suicide, other</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td>fAM</td>
</tr>
<tr>
<td>556.</td>
<td>Mental illness: schizophrenia</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td>fAM</td>
</tr>
<tr>
<td>60.</td>
<td>Neuromuscular diseases</td>
<td>Poster</td>
<td>mAM</td>
<td></td>
<td></td>
<td></td>
<td>fAM</td>
</tr>
<tr>
<td>454.</td>
<td>Neurotoxicity: amino acids</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td>fAM</td>
</tr>
<tr>
<td>187.</td>
<td>Neurotoxicity: metals</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td>fAM</td>
</tr>
<tr>
<td>515.</td>
<td>Neurotoxicity: MPTP</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td>fAM</td>
</tr>
<tr>
<td>455.</td>
<td>Neurotoxicity: other I</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td>fAM</td>
</tr>
<tr>
<td>251.</td>
<td>Neurotoxicity: PNS and retina</td>
<td>Poster</td>
<td>tuAM</td>
<td></td>
<td></td>
<td></td>
<td>fAM</td>
</tr>
<tr>
<td>256.</td>
<td>Recapitulation of Developmental Mechanisms in Neurodegenerative Disorders</td>
<td>Symp.</td>
<td>wAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>206.</td>
<td>Trauma</td>
<td>Slide</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>319.</td>
<td>Trauma: brain injury</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>552.</td>
<td>Trauma: spinal cord, NMDA and other</td>
<td>Poster</td>
<td>tuPM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Other:**

| 325.           | NINDS: Forty Years of Progress | Symp. | wPM  |      |      |      |      |

To examine the psychobiological influences on performance rhythms, this study determined the role of working memory in daily performance of rhesus monkeys. We recorded the effects of acetylcholine on performance at two different times of the day. Scopolamine injections were given at 1 and 5 hours after lights-on. We used doses reported to affect working memory selectively.

To earn their food, monkeys had to perform a vigilance task followed by a color-pair discrimination task. Trials were delivered on the average of every 2.4 min, and response latencies and choices were recorded. We assayed vigilance performance after the scopolamine injection. This phase lasted longer after the morning dose than in the afternoon and their performance recovered sooner in the afternoon.

Normally, these monkeys made only 1 to 3 discrimination mistakes per day. We compared performance for the 2 hrs following scopolamine with the comparable time period in baseline no-drug- and saline-injected conditions. The monkeys made 5-15 times more mistakes when given scopolamine (p < 0.001). The monkeys made nearly twice the number of mistakes in the morning (6.79) than they did in the afternoon (3.52) following a scopolamine injection (p = 0.02).

The time of day differences in mistakes under the influence of scopolamine suggests that working memory is more susceptible to disruption in the morning. This pattern of susceptibility might be adaptive if we assume that natural disruptions of memory, such as fatigue, are more likely to occur in the afternoon when the animal is resistant to such disruptions. Supported by VA Research.

EFFECTS OF SCOPOLAMINE AND CLONIDINE ON RECOGNITION MEMORY IN Rhesus Monkeys With Bilateral Basal Forebrain Cholinergic System Lesions. C. Chavira, T. Alpergil, M. Ortega*, and M. Maldonado. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

Both acetylcholine (ACh) and serotonin (5HT) are thought to play critical roles in processes of memory. Recent evidence suggests that NE may influence the activity of ACCh neurons in the basal forebrain (BF). To investigate the neurochemical contributions of these 2 neurotransmitters, we administered the muscarinic and alpha 2-adrenergic antagonists scopolamine (SCOP) and the alpha 2-adrenoceptor agonist, clonidine (CLON). In addition, we had monkeys to analyze EEG power density in the delta, theta, and alpha bands to determine if the drug administrations altered the measuring times.

We observed that scopolamine, alone, demonstrated a significant increase in the alpha band power density of the EEG recorded on the right side of the monkeys. Clonidine also increased the alpha EEG power density of the EEG recorded on the right side of the monkeys. The combination of SCOP and CLON had no additive effects on the EEG power density compared to the effects of SCOP or CLON alone.

In conclusion, the results of this study suggest that scopolamine and clonidine affect the activity of ACCh neurons in the basal forebrain. These results are consistent with recent findings in the literature that suggest a role for NE in the regulation of ACCh neurotransmission.

Although these results are preliminary, they provide evidence for the potential role of NE in the regulation of ACCh neurotransmission and suggest that further investigation is warranted.

SCOPOLAMINE IMPAIRS COGNITIVE FUNCTION IN ANIMALS AND HUMANS, When administered systemically. Since scopolamine has profound effects on peripheral and central cholinergic function, we developed a convenient, refillable system for continuously administering compounds to the central nervous system of rhesus monkeys.

Two eight-year-old rhesus monkeys were implanted with a subcutaneous infusion pump connected to a cannula stereotaxically directed toward the right lateral ventricle. Continuous ICV infusion produced a dose-dependent decrease in the number of responses. The magnitude of the response decrement produced by scopolamine was dependent on the stimulus duration with fewer responses occurring at the shorter stimulus durations. This decrease in performance was also time dependent and occurred mainly during the last half of the test session.

These data suggest that scopolamine produces a deficit in sustained attention that is mediated through the central cholinergic blockade in the rhesus monkey. These procedures were well tolerated and the system remained functional over an extended time period.

SINGLE DOSE PHARMACO-EEG PREDICTS RESPONSE TO TETRAHYDROAMINOCINORIDINE TREATMENT IN ALZHEIMER'S DISEASE. B. Schling*; K. Alahouman*; J. Partanen*; and P. Riekkinen*. Dept. of Neurology and Clinical Neurophysiology, University of Kuopio, P.O.Box 6, 70211 Kuopio, Finland.

Only 30-50% of Alzheimer (AD) patients benefit from various cholinomimetics such as tetrahydroaminoacridine (THA). Because we recently found that progressive EEG decline is probably related to cell damage of the nucleus basalis of Meynert, it was reasonable to assume that THA will help to identify responsive AD patients. We selected 12 AD patients (NINCDS-ADRDA criteria) and 7 controls with AMY (age associated memory impairment) without time dependent and occurred mainly during the last half of the test session.

In responders alpha-delta ratio increased 57.6% and alpha-theta ratio increased 61.4% compared to baseline. In nonresponders alpha-delta ratio and alpha-theta ratio decreased 33.3% and 10.7%, respectively. The AMY group showed no change in these parameters. These results suggest that a single dose pharma-EEG predicts treatment response to THA.

MU-OPIOID AGONIST AUGMENTS LOCOMOTOR ACTIVITY AFTER BILATERAL 6-HYDROXYPYRIDINE LESIONS IN THE NUCLEUS ACCUMBENS WITHOUT INVOLVING RECEPTOR UPREGULATION. L. Churchill and P.W. Kalivas. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

The mesolimbic dopamine system was lesioned by bilateral injection of 6-hydroxypyrine into the nucleus accumbens of rats. Ten days or more after the lesion, locomotor activity increased to a significantly greater extent after microinjection of the /3-opioid agonist, Tyr-D-ala-Gly-met-Phe-Gly-Oh (DAGO). A much smaller lesion-induced increase was observed after the /3-agonist, D-phenylcarbamate (DPCPREP).

A receptor autoradiography with [3H]DAGO or [3H]DPPD binding to the opioid receptors showed no significant differences between control and lesioned rats at 15 days after the lesion. Forskolin (1 /M) stimulated adenylyl cyclase to a greater extent in the lesioned rats than in the sham treated rats (ascorbic acid treatment), but the opioid inhibition of forskolin stimulation did not differ between controls and lesioned rats. DAGO appeared more effective than DPPD in inhibiting forskolin-stimulated adenylyl cyclase. In conclusion, the behavioral analyses argue for a greater upregulation of the /3-opioid induced motor activity than that of the /3-agonist after dopamine depletion. The mechanisms underlying this augmentation remain to be caused by increase in opioid receptor binding or inhibition of adenyl cyclase.

NEUROPEPTIDES AND BEHAVIOR III


Studies using mammals indicate that corticotropin-releasing factor (CRF) may coordinate physiological, autonomic and behavioral responses to stress stimuli. We present evidence that CRF is involved in regulating stress-induced behavior in amphibians and further that the behavioral effects of CRF are modified by the opioid system. Interpreting ICV injections of CRF dose-dependently stimulated locomotor activity in male rough-skinned newts (Taricha granulosa). This effect of CRF was completely blocked by the CRF receptor antagonist, naloxone (N), which indicates that CRF acts through an opioid receptor. Exposure to stressful environmental stimuli (either warm water or a 30-s period of handling) also stimulated locomotor activity. Injections of CRF did not produce a stress-induced locomotor activity regardless of the nature of the stressful stimulus.

Studies with opioid agonists and antagonists suggest that CRF and opioids interact to control locomotor activity in T. granulosa. The opioid μ-receptor agonist, morphine, at a dose that did not affect spontaneous locomotor activity, suppressed CRF-induced locomotor activity in a naloxone-reversable manner. In contrast, the preferential opioid kappa receptor agonist, bremazocine, which dose-dependently suppressed spontaneous locomotor activity, did not affect CRF-induced locomotor activity. Experiments using the opioid receptor antagonist, naloxone, found that naloxone had no effect on spontaneous locomotor activity but when co-injected with CRF, completely blocked the effects of CRF on locomotor activity. Although the effect of naloxone on CRF-induced locomotor activity is opposite to that typically observed in mammals, our other results are consistent with observations in mammals. Thus the neuroendocrine mechanisms controlling stress-induced locomotor activity appear to be evolutionarily conserved among vertebrates.
375.3 NEUROTENSIN/ADENOSINE INTERACTION IN CNS. F. Pelletier and R. F. Butterworth, Lab. of Neurochemistry, CRC A-V, Hôpital St-Luc, Montreal, Que., Canada.

375.4 ASTROCYTIC BENZODIAZEPINE RECEPTORS AND THEIR ENDOGENOUS LIGANDS IN HEPATIC ENCEPHALOPATHY. J. P. Gigure, J. Lavoie, M. rogue, J.-D. Desy, H. Vaudry, G. Pelletier and R. F. Butterworth, Lab. of Neurochemistry, CRC A-V, Hôpital St-Luc, Montreal, Que., Canada.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
375.10


A crustacean eyestalk is a putative circadian neurosecretory pacemaker. However, its capability to maintain a self-sustained circadian rhythm in vitro has not been demonstrated. In this work we report the persistence of a circadian rhythm in the isolated eyestalk of the crayfish Procambarus clarkii.

As a marker of neurosecretion we used the red pigment concentrating hormone (RPCH) which was quantified. a) by immunoenzymatic assay (ELISA), with an antibody raised in our laboratory. b) by HPLC, and c) by bioassay on isolated crayfish epithelium. Isolated eyestalks were kept in organ culture during the experiment, in 20% Leibovitz medium, with 1% fetal calf serum. While in culture, they were subjected either to continuous darkness (0:0), cycles of 12:12 hrs. of light and darkness (L:D), or continuous light (L:L). In isolation the RPCH amount in the eyestalk varied in a circadian manner within a range of 2x10^{-10} to 8x10^{-10} gr, with maximum during night phase. t = 25.5 ± 1.5 L.D.; and 25 ± 1 in D.D.

375.11


Egglaying in Anaxis californica is seasonal, occurring mainly in the summer, and is caused by a peptide egg-laying hormone (EILH) released during a discharge by the bag cells. Alpha bag cell peptides (α-BCP), one of the peptides released along with EILH is secreted to play an autoregulatory role in modulating the characteristics of the discharge. The feedback effects of α-BCP have been reported as autocrine by some and autocrine/autobitory by others, in experiments performed at 14°C and room temperature respectively. These temperatures approximate winter and summer water temperatures experienced by the animal; thus the effect of α-BCP may be temperature-dependent and may be a factor in determining the seasonality of egg-laying. To test this possibility, α-BCP's effects on bag cell membrane potential was determined. At 15°C, α-BCP caused a hyperpolarization, whereas at 20°C, the peptide caused a small depolarisation. Extracellular recordings revealed that at 15°C, α-BCP shortened the duration of the bag cell discharge and decreased the total number of action potentials generated. At 20°C, the peptide lengthened the discharge and increased the number of action potentials. A transient rise in CAP is associated with the discharge; therefore, we measured α-BCP's effect on bag cell CAP levels at various temperatures throughout the animal's natural environmental range (12°C-25°C). The influence of α-BCP is highly temperature-sensitive. The function of the peptide reversed from inhibition (12-13°C) to stimulation (17-25°C) over a 2.5°C range. These results imply that α-BCP is temperature-sensitive, functioning in an autocrine/autobitory manner at 15°C and becoming autocrine at 20°C. Thus, it may function in coordination with other factors to control egg-laying in response to seasonal temperature variations.

376.1

Histidine, Histamine, and Amino Acid Neurotransmitter Dynamics of Hypothalamic Nuclei in Meal Fed Rats. Dodda, S.J., L.P. Mercer* and J.D. Dunn, Dept. of Biochem. Oral Roberts Univ., Sch. of Med., Tulsa, OK 74112. Neurotransmitter patterns are assessed under regimented meal conditions and compared to previous findings of elevated histidine in protein energy malnutrition. Quantitative experiments were performed on a well-housed rat. Gli, tau, ADB, Glu, Asp, levels of hypothalamic nuceli, in relation to meal fed rats. In meal regressed rats, glutamate in the lateral hypothalamus (LH) and ventromedial hypothalamus (VMH) are inversely related. Over the course of the meal, high VMH his and low LH his reflect hunger, and low VMH his and high LH his correlate with satiated states. To contrast no significant dynamics are demonstrated with HA, however VMH HA decreases with increasing hunger in the premeal (ac) period. Median Emicence/Arcuate nuclei (EM) reveal increasing HA with increasing satiety. The paraventricular nucleus (PVN) was found to be empty of HA in ac period. Levels fall significantly during the digestive phase and return to ac levels 1 hour postmeal. Putative amino acid neurotransmitter dynamics were also found to correlate anatomically with food intake changes.

Data is obtained by microdissection of freeze-dried brain sections, obtained at appropriate time intervals. Amino acid analysis is done by HPLC methodology and HA levels are obtained with a radioimmunoassay.

376.2

INDEPENDENT INGESTION OF WATER AND MILK BY PRE-WEANING RAT PUPS FOLLOWING SYSTEMIC HISTAMINE. Steven M. Specht, Robyn M. Cashmore* and Kevin B. Dampsey*. Psychology Department, Lebanon Valley College of Pennsylvania, Annville, PA 17003.

An independent ingestion paradigm, in which pre-weaning rat pups are allowed to consume fluid from the floor of a test chamber, was used to assess intake levels in 13-day-old rat pups. Each pup received an injection of either 1) 0.15M NaCl, 2) 1.0M NaCl, 3) 40 mg/kg histamine (HA) or 4) 80 mg/kg HA and was placed in a test chamber (35°C) for 30 min with either water or milk spread on the floor. Although pups exhibited increased water intake following hypertonic 1.0M NaCl, there was no apparent increase in water intake following either dose of HA. Pups in all treatment groups exhibited similar milk intake levels during the 30 min test. These results suggest that 1) the independent ingestion procedure may be useful to examine water intake following homeostatic challenges in pre-weaning rat pups, and 2) there may be a relative insensitivity to the dipsogenic effects of HA for pups at this age tested with this procedure. These results are consistent with the finding that weaning rats are less sensitive than adults to the dipsogenic effects of HA, requiring higher doses of HA than adults to elicit drinking (20 vs. 5 mg/kg, respectively). (Soc. for Neurosci., S.M. and Spear, L.P., Physiol. Behav., 45:63, 1989).
CLONIDINE DIFFERENTIALLY AFFECTS MACRONUTRIENT INTAKE IN GENETICALLY OBESE AND Lean MICE: DOSE EFFECT RELATIONSHIPS. P.J. Currie and L.M. Wilson. Psychol.Dept., Washington, Montana. Clonidine (CLON), the α2-noradrenergic agonist, reduces total energy intake primarily through suppression of carbohydrate (CHO) and fat intakes. Both carbohydrate and fat intakes are directly related to α2 receptors in the ventromedial nucleus (VMN) and lateral hypothalamic (LH) of male rats only. Circulating glucose is positively related to α2 receptors in the VMN, LH and noradrenergic innate related to α2 receptors in the VMN. These results reveal a close relationship between nutrient intake and appetite, circulating hormones and α receptors in these hypothalamic sites.

MECHANISM OF TYROSINE (TYR) POTENTIATION OF MIXED-ACTING SYMPATHOMIMETIC ANOREXIGENS (MASA). K.M. Bull and T.J. Maher. Dept. Pharmacol. Mass.Coll. Pharm., Worcester, MA. We previously reported a TYR specific potentiation of the MASA [(D,L)-amphetamine] (PAPA), (+)-amphetamine and (-)-ephedrine (EPH). Thus we examined the dependence on functioning TYR hydroxylase (TYR), brain TEA and the rate of catecholamines (CA) in this potentiation. To determine if the potential of PAPA required ligand-mediated conversion for CA synthesis, we pretreated with α-noradrenaline and then administered EPH (20mg/Kg) with or without TYR (200mg/Kg). OMP blocked both the anorectic activity of EPH and that of TYR in food-deprived rats. To determine the requirement for central TYR we administered equicaloric valine (VAL), which competes with TYR for uptake into brain, to food-deprived rats, TYR, or VAL+TYR with either saline (SAL) or PAPA (20 mg/Kg). Neither VAL, TYR or VAL+TYR altered food intake when administered to SAL pretreated rodents. While VAL alone failed to alter PAPA-induced anorexia, VAL+TYR attenuated the normally observed pot-antion by TYR. We also administered two add-itional MASA:(-)-norepinephrine, (+)-pseudoephedrine, and two direct-acting agonists: mescaline and methoxyphenamine. Only the MASA were potentiated by TYR. These results suggest that TYR's potentiation results from a central action involving increased CA synthesis via TH.

OPPOSITE EFFECTS ON INGESTIVE BEHAVIOR FOLLOWING CHRONIC MUSCIMOL ADMINISTRATION INTO THE MEDIAN RAPHE OR VENTRAL TRIGEMINAL AREA. T.R. Strafand and D. Wirtshafter. Dept. Psychology and Committee on Neuroscience, University of Illinois at Chicago, Chicago, IL, 60680. Previously, we have demonstrated large increases in food and water intake in rats during the hour following acute microinjections of GABA-A agonist muscimol into the median raphe (MR). Smaller increases were observed following similar injections into the VTA. In order to assess the effects of chronic administration of muscimol into these brain areas, L5 rat osmotic minipumps were attached to injector cannulae terminating in either the MH or the VTA and were implanted either anesethic. The muscimol was administered in a dose of 25mg/0.5ul at an infusion rate of 0.5 ul/hour. Animals receiving the MR infusions exhibited extremely large increases in water intake and, to a lesser extent, food intake, along with a severe disruption of the normal meal frequency. Hyperdipsia and hyperphagia appeared within a few hours of the minipump implantation and continued to be present in 75% of the cases. Peak 24 hour water intakes were in the 280-350 ml range with food intakes in excess of 40 g for the same period. In contrast, chronic infusions of muscimol into the MR exhibited increased meal patterning and total intake in the rat.
376.9 THE EFFECT OF INTRAPERITONEAL ADMINISTRATION OF IC5 203-930 AND QUANTERIZED IC5 203-930 ON FOOD INTAKE OF RATS FED AN AMINO ACID IMBALANCED DIET.
Dept VM/Physical Sci and Food Intake Lab, Univ. Calif., Davis, CA, 95616.
We have previously reported that the anorectic response of rats to an amino acid imbalanced diet (IMB) is blocked by intraperitoneal (ip) injections, but not intracerebroventricular injections of the [SH]-agonist IC5 203-930 (ICS). ICS has been shown to cross the blood brain barrier (BBB), while quanterized ICS (Q-ICS) should not. Since evidence indicates that the IMB response to ICS is more centrally than peripherally, we used ip administration of ICS and Q-ICS to determine whether the SH7 receptors that are involved in the rats' response to IMB are located centrally or peripherally. Male Sprague-Dawley rats (n=7 groups) were permitted to consume a low protein basal diet for 10 days. Rats received ICS, Q-ICS, (7.8 or 28.1 mg/kg body weight) or saline within 30 min of onset of the dark cycle, and were then fed IMB. Food intake was recorded after 3, 6, 12, and 24 hr. From 0 to 6 hr, rats that received ICS and Q-ICS injections are similar amounts of IMB (P>10). Rats injected with 28.1 mg/kg ICS and Q-ICS are significantly more IMB (P<0.01) from 6 to 24 hr than rats injected with 7.8 mg/kg of these drugs, demonstrating a dose-dependent response to these SH7 antagonists. There were no significant differences (P>10) between ICS and Q-ICS in IMB intake. If Q-ICS does not cross the BBB, these results suggest that a major portion of the effect of ICS in blocking the anorectic response to IMB is mediated peripherally. Supported by NIH DK 07535, USDA CRCS 1-2418, CNRU 5K747 and a gift from Sandoz Research Institute.

376.11 HYPOTHALAMIC EFFECTS OF 5-HYDROXYTRYPTOPHAN ON FEEDING IN HUNGRY RATS. C.T. Tral. Department of Bioloay, National Changhua University of Education, Changhua, Taiwan, ROC
The effects of the serotonin precursor, 5-hydroxytryptophan (5-HTP) on food intake in free-feeding and food deprived rats were examined. In free-feeding, 5-HTP (100 mg/kg i.p.) reduced food intake by 86.3%. The anorectic effect of 5-HTP was antagonized by cyproheptadine (CYP, 4 mg/kg, i.p.), a serotonin blocker. However, following food deprivation, the food intake was increased 24.7% in 5-HTP treated rats. The food intake was decreased 42.8% in 5-HTP treated rats. That is, the feeding of hungry rats was still inhibited by 5-HTP. Nevertheless, this inhibition of 5-HTP on food intake in hungry rats was almost recovered by CYP. The evidences indicated the anorectic action of 5-HTP in free-feeding and in hungry rats were mediated by serotonergic mechanism. As to analysis of meal patterns, the 5-HTP treated hungry rats ate only 0.16 gms in the initial 2 hr session and took 2.7 gms in the later 6 hr period, while the saline controls ate 10.3 gms in the initial 2 hr session and 2.1 gms in the later 6 hr period. This opposite result apparently showed that the effect of 5-HTP on feeding was inhibition of the initial phase of hungry period. These evidences are in favor of a conclusion that there is an existence of a serotonergic mechanism in brain to be inhibitory for feeding via inhibition of hunger.

There is evidence that the appetite suppression which is an apparent cause of TCDD-induced death, may be a result of serotonergic abnormalities followed by an elevation of serum and hypothalamic tryptophan levels and finally resulting in a serotonin (5-HT)-mediated reduction of food intake. Consequently, depletion of central 5-HT should alter the TCDD-induced starvation syndrome. Central 5-HT depletion was accomplished by using i.c.v. infusions of the serotonin 5-HT-2 antagonist ketanserin (5-HT-PDF 150 g/kg of TCDD) containing 0.1% acetic acid. Rats (n=5) were pretreated with desipramine (25 mg/kg i.p.) 30 min. before i.c.v. infusion. Two weeks after infusion these rats and a group of lesioned rats (n=3) were treated with a lethal dose of TCDD (125 mg/kg i.p.). These naive control rats were injected with vehicle alone (corn oil, 4 ml/kg i.p.). All rats were monitored daily for food intake and body weight development. On day 13 after treatment all rats were sacrificed and 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) dopamine (DA) and its metabolite dihydroxyphenylacetic acid (DOPAC) were quantitated in the hypothalamus using HPLC with electrochemical detection. In 2 of 5 of the 5-HT7-depleted animals hypothalamic 5-HT was depleted more than 90% and 5-HIAA more than 85% compared to control animals. In the remaining 3 animals depletions were between 30% and 61% for 5-HT and 36% and 57% for 5-HIAA. No alterations were found in the hypothalamic levels of DA and DOPAC. No differences were observed regarding body weight development and food intake in TCDD-treated rats with or without central depletion of 5-HT. These results suggest that although TCDD increases central 5-HT levels as a result of increased plasma tryptophan, this may not be the sole cause for reduced food intake and lethality. In further studies it needs to be determined whether elevations in periphereral tryptophan and/or 5-HT can cause TCDD induced starvation.

Male Han/Wistar rats given a sublethal dose of TCDD (1000 μg/kg ip in corn oil) showed disturbed metabolic control of food intake and lowered body weight for months after TCDD treatment. The turnover of CAs was accelerated about 20-80% in almost all brain areas in the TCDD group. These results support our previous findings suggesting only a minor or secondary role for the brain monoamines in TCDD toxicity.

376.14 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ENHANCES RESPONSIVENESS TO SATIETY SIGNALS AND CAUSES PERSISTENT ALTERATIONS IN RESPONSES TO FEEDING REGULATORY CHALLENGES IN HAM/ WISTAR RATS. R. Pohjanzristi* and J. Topstato. Natl. Public Health Instit., Dept. of Environ. Hyg. and Toxicol., P.O.B. 95, SF-70701, Kuopio, Finland.
A single sublethal dose of TCDD (1000 or 3000 μg/kg ip) led to persistent hyperactivity and retardation of growth in Ham/Wistar rats. In one-bottle tests, these rats showed diminished sucrose drinking accompanied by a progressive tendency toward increased saccharin consumption over control levels. In contrast to control rats, they did not display hyperphagia in response to cellular glucopenia by 2-deoxyglucose and reduced their feed intake after inhibition of fatty acid oxidation by mearcaptoacetate. Their feeding behavioral responses to insulin and naloxone were attenuated. Paradoxically, their autonomic activity (i.e., heart rate) was increased and noninterference activity was suppressed following administration of diazoxide and (even more so) fructose significantly suppressed starvation-induced eating in TCDD-treated rats while being without effect in controls. These findings suggest a role of regional glucose levels for brain neurotransmission and stimulation of the hypothalamus to modulate brain glucose activity. Furthermore, the modulation of TCDD-induced GLUCOSE RESPONSES TO SATIETY SIGNALS COULD BE CRUCIAL UNDERLYING THE PERMANENTLY SUBNORMAL BODY WEIGHT MAINTAINED BY TCDD-TREATED RATS.
376.15

The D-2 antagonist, raclopride, and the D-1 antagonist, SCH 23390, inhibit intake of 10% sucrose in 14-day-old rat pups when a pup ingests 10% sucrose by licking it from the floor of a tissue-lined beaker (Independent Ingestion), II, but not when a pup ingests 10% sucrose infused into its mouth through an anterior, sublingual, oral catheter (OC; Smith et al, 1989; Tyrka et al, this meeting).

The differential potencies of the agonists may be due to a differential effect of the ingestion of 10% sucrose on central dopaminergic (DA) activity in the two tests.

Method: To test this hypothesis, DA, DOPAC and HVA were measured by HPLC-EC in 14-day-old, male and female pups at the end of a 20-min II or OC test.

Results: Ingestion of 10% sucrose by II, but not by OC increased DOPAC/DA in hypothalamic and olfactory tubercle (p<.05). Both II and OC increased caudate DOPAC/DA (p<.05). In contrast, ingestion of 10% sucrose by OC increased HVA/DA in amygdala and hypothalamus (p<.05), but II of 10% sucrose had no effect on HVA/DA in any region.

Conclusions: (1) Regional pattern of DA metabolism depends on the mode of ingestion (II or OC) of 10% sucrose; (2) increased DOPAC/DA in hypothalamic and olfactory tubercle by II, but not OC, correlates with the inhibitory effect of raclopride and SCH 23390 on II, but not OC; (3) the significant increase in hypothalamic DOPAC/DA produced by II of 10% sucrose in 14-day-old pups extends our observation of this in adult rats (Smith et al, 1987) and suggests that this effect is innate rather than acquired.

Supported by MH51455 and MH800149 (GPS).

376.16

SCH 23390, a D-1 dopaminergic antagonist, decreases the sham feeding of sucrose in adult rats (ID50=60 μg/kg, ip, Schneider et al, 1988). To determine the degree of this effect, intake of 10% sucrose was measured at postnatal days 7, 14, and 21. Pups were removed from the litter for 4h and then placed in a warm, humid chamber for one of two 20-min tests: (1) continuous infusion of 10% sucrose through an anterior, sublingual catheter (Hall, 1979) or (2) independent ingestion of 10% sucrose from the bottom of a test beaker. A dose of SCH 23390 (20-267 μg/kg) or saline alone was injected 15 min prior to an intake test. Each pup was tested only once. SCH 23390 was significantly more potent for decreasing intake in the independent ingestion test than in the intraoral infusion test at each of the three ages (Table).

<table>
<thead>
<tr>
<th>POTENCY OF SCH 23390 (ID50 μg/kg, ip)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
</tr>
<tr>
<td>14</td>
</tr>
<tr>
<td>21</td>
</tr>
</tbody>
</table>

Although the reason(s) for the differential potency in the 2 tests is not clear, the results suggest that D-1 receptor activity is necessary for the positive reinforcing effects of sucrose on independent eating as early as postnatal day 7. In contrast, D-2 receptor activity is not necessary for independent ingestion until day 14 (Smith et al, 1989).

Supported by NIMH MH51455 and NIMHD RSA MH00149.

376.17

Changes of sham feeding are often assumed to reflect alterations of palatability. However, sham feeding is influenced by many performance variables independent of palatability. We adapted the curve shift paradigm, used in the brain stimulation reward field to distinguish reward from performance effects, to sham feeding to discriminate manipulable variables which alter palatability from those which influence sham feeding performance independent of any influence on taste.

Adult rats sham fed sucrose solutions ranging from 0.3M to 1.5M. Concentration-intake functions were generated which plotted sham intake (in ml/30 min) versus log of sucrose concentration. Curve shifts were indexed by calculating the log sucrose concentration which produced 50% of asymptotic sham intake, in the half-max concentration. Decreasing sucrose palatability by quinine adulteration reduced asymptotic intake by 27% and shifted the concentration-intake function to the right. Half-max values were .89 for unadulterated, and 1.24 for adulterated, sucrose. Dopamine agonism (5 mg/kg pimozide) mimicked effects of degrading palatability-reduced asymptotic sham intake with a rightward shift of the concentration-intake function. Pinacidil increased half-max sucrose concentration from 7.0 to 1.1. In contrast, rats sham feeding after 1, 6, 12, or 18 hrs food deprivation showed a two-fold change in asymptotic sham intake but no shift in the concentration-intake function; half-max sucrose concentrations were identical at all deprivation levels. These data suggest that: i) by effects of a treatment on sham feeding must be assessed by examining the entire concentration-intake function, and ii) congenial with the conclusion from analogous studies in the brain stimulation reward literature, although many variables alter sham feeding performance, only these treatments which curve-shift operate by altering the motivating capacity of taste.

376.18

In an attempt to determine whether the effects of pimozide and amphetamine on the microstructural components of the first meal following 24 hour food deprivation in rats could be explained by changes in dopaminergic function, we investigated the effects of systemic pimozide and amphetamine on the microstructural components of the first meal following 24 hour food deprivation in rats. Deprived subjects were adapted to a two-trial paradigm, with weights of wet mash (Purina rat chow and water, 3:2 w/w) and injections of physiological saline over 11 days. Each animal served in all conditions. Experimental sessions consisted of a maximum of 30 feeding trials, each of 30 sec. duration, separated by a 90 sec. intertrial interval. Food intake was measured following each trial, and bites and pauses were measured on-line within each trial. Sessions were videocoded for subsequent verification of latency; duration; and bite frequency and distribution.

Consistent with previous work in our laboratory, all groups showed a decline in intake rate over the course of a session that was a function of decreasing bite size; bite frequency remained constant over trials. In addition, pimozide (0.5 and 1 mg/kg i.p.) and amphetamine (0.5 and 1 mg/kg i.p) each decreased cumulative intake in a dose-dependent manner, by both reducing amount eaten per trial and decreasing duration of eating per session, compared to vehicle controls. Pimozide also decreased number of trials on which eating occurred, leaving duration of eating per trial unaffected. Therefore, pimozide reduced amount eaten per trial by slowing local rate of intake. By contrast, amphetamine reduced intake per trial by decreasing duration of eating within 30 sec. trials, either by increasing latency or pausing, and not by altering intake rate. The basis for pimozide's reduction of local intake rate is apparently a decrease in bite size, because there was no alteration of local bite rate.

376.19

Dopamine (DA) is thought to be critically involved in motor and motivational aspects of behavior. In particular there is considerable evidence that DA may mediate certain components of feeding behavior. Both neuroleptics and amphetamine disrupt feeding, but little is known about the specific striatal subregions involved in these effects. Food-deprived rats were injected with either haloperidol (0.025, 0.25, 2.5 μg) or amphetamine (1, 10, 20 μg) in one of three striatal sites: nucleus accumbens (N.Acc.), ventral lateral striatum (VLS), and dorsolateral striatum (DLS). It was found that food intake, feeding rate, food spillage and feeding durations were differentially affected following drug administration into N.Acc., VLS, and DLS. Injections into DLS produced no significant changes in ingestive behaviors. Results were found to be compatible with previous work that indicates a functional heterogeneity of the striatum. Moreover, it is hypothesized from these results that VLS is specifically involved in the regulation of oral motor behavior while that N.Acc. may regulate attentional or switching mechanisms that are required for the maintenance of feeding behavior.
377.1
EFFECT OF LITHIUM CHLORIDE AND DESIPRAMINE ON HIV REPLICATION. D. L. Evans, M. S. Smith, J. Pettitt, R. N. Smiley, Dept. of Psychiatry, University of Colorado, Boulder, CO, 80309.

Lithium and tricyclic antidepressants are used for the treatment of affective disorders in patients with HIV-related illness. Because lithium has immunomodulatory properties, we studied the effects of lithium chloride (LiCl) and the antidepressant, desipramine (DMI), on HIV replication order to determine if these drugs can demonstrate an anti-HIV effect or conversely, an enhancement of HIV replication.

We studied the effect of LiCl and DMI on HIV replication in T-lymphocytes in culture, and the effect of LiCl on reverse transcriptase activity in vitro. HIV and C3 cells were infected with the LAV-1 strain of HIV-1 and the production of HIV from drug treated cells was estimated by reverse transcriptase activity.

We found no effect of LiCl (20mM - 200mM) on the activity of HIV to replicate in T-cells, as well as no effect of DMI (10 ng per ml - 1 mg per ml) on virus replication. In addition, we found no effect of LiCl (1 mM-20mM) on the reverse transcriptase enzyme itself.

These preliminary data suggest that neither lithium, nor the antidepressant, DMI, diminish HIV replication. Thus, these findings do not support an anti-HIV effect for lithium or DMI.

However, and importantly for the clinical management of individuals with HIV infection, this potential no-enhancement of HIV replication and therefore do not claim the question of these agents for the treatment of affective illnesses in these individuals.

377.2

Opirompl is a tricyclic antidepressant that has a structure which is very similar to, but macro clinically effective antidepressants. Though its structure is similar to other antidepressants, and opipramol itself has been used extensively as an effective antidepressant medication, it is virtually inactive when assayed for inhibition of catecholamine uptake (a mechanism through which many antidepressants act, but which opipramol does not affect their therapeutic effects). We have used tritium labeled opipramol to study its high affinity binding to brain membranes. [3H]Opirompl labels two sites in brain membranes for which it has equal high affinity (kD-1-4nM, Total Bmax 5 nmol/mg protein). Haloperidol, which has very high affinity for sigma receptors, differentiates these two sites clearly. Haloperidol has a KI of 11mM for the sigma receptor component, and [3H]Opirompl binds with a KI of 350mM for the novel opipramol binding site. Therefore, we have blocked the sigma component with 50mM haloperidol to study this novel site in isolation. Pharmacological characterization has confirmed that [3H]Opirompl labels the sigma receptor and that the second binding site is not any known neurotransmitter receptor. Also, several drugs which are relatively inactive in inhibiting catecholamine uptake and yet active as antidepressants are potent inhibitors of opipramol binding to this novel site. We suggest that this site may mediate some of the antidepressant effects of these drugs.

377.3

We previously reported that chronic antidepressants produce a decrease in tyrosine hydroxylase (TH) mRNA and in TH immunoreactivity in locus ceruleus (LC) but not in substantia nigra (SN) (McMahon et al., Soc. Neurosci. Abst. 15:986, 1989). To further investigate this finding we measured the enzymatic activity of TH (assayed in vitro) in several regions of rat brain after chronic imipramine treatment. In the dopamine cell body regions, TH activity was decreased by 20% in SN (A9) and by 45% in ventromedial orbital area (A10). In an analogous fashion, TH activity was decreased by 20-40% in projection regions of dopamine A10 neurons, i.e. nucleus accumbens and medial prefrontal cortex, and by 30-60% in substantia nigra, an area which receives projections from both A9 and A10 cell body regions. Despite previously observed decreases in TH immunoreactivity and mRNA in LC, no significant decrease in LC was found in LC, the noradrenergic cell body region, or in the hippocampus, which receives projections from LC. These results demonstrate that TH activity is diminished in dopamine neurons by chronic imipramine and support a growing body of evidence that antidepressants also alter dopaminergic function. It remains to be seen whether the effects on TH in dopamine regions are relatively more selective for dopamine A10 vs. A9 neurons and whether other noradrenergic regions are affected. (Supported by USPHS MH45481 and MH14092).

377.5

We have reported that exposure of rats to three sessions of repeated inescapable shock induces a long-lasting period of decreased daily running wheel activity, which is antagonized by chronic administration of desipramine following the stress sessions (Deans, Sibert and Meier, Pharmacol. Physiol. Behav. 30(1985):1-29). In the present study we administered adinazolam 30’ prior to each stress session (5 and 10 mg/kg, IP) or in the days after the stress sessions (5 and 20 mg/100 ml in drinking water). Adinazolam, both attenuated the stress-induced decrease in running wheel activity when given prior to stress, and reversed it when given after stress. As in the case of desipramine, the effect of adinazolam required 7 days for maximum effect, produced a significant increase in normal levels and showed a strong curvilinear dose response curve. Adinazolam in another pattern of administration had minimal effects on the activity of untraumatized animals. In general, anxiolytic agents acutely administered prior to stress reduce the effects of inescapable stress such as learned helplessness (Deans, et al., Psychopharmacol. Bull., submitted). The effects of i.v. administered adinazolam were assessed on the spontaneous firing of putative noradrenergic neurons in the locus coeruleus (LC) of male Sprague-Dawley rats anesthetized with chloral hydrate. Standard extracellular single-unit recording techniques using glass microelectrodes were employed. Although considerable variability was observed, overall, all trazodone increased the firing rate of noradrenergic LC neurons in a dose-dependent manner when assessed between both cells and within cells. The effects observed produce an increase in firing rate of 25% (ED50) was calculated to be 0.128 mg/kg, i.v., and the ED70=0.659 mg/kg, i.v. These results agree generally with those of Horell and Dresse (1982, Arch. Int. Pharmacodynamy., 260, 299-310), who also observed potent inhibition of serotonergic dorsal raphe neurons with trazodone. The inhibition of serotoninergic neurons and mild excitation of noradrenergic neurons defines a unique electrophysiological profile for trazodone when compared to other antidepressants.
377.7 RAPID TRYPPTOPHAN DEPLETION REVERSES ANTIDEPRESSANT RESPONSE AND ALLOWS MOOD INPRESSION (P. Delgado, D.S. Charney, L.J. Price, G.K. Aspleghan, G.S. Haninger, West Haven VAMC and Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06510). Brain serotonin (5-HT) content is dependent on plasma levels of the essential amino acid, tryptophan (TRP). We have previously reported on the effects of rapid dietary TRP depletion in patients with depression and have extended those reports and further characterized the effects of rapid TRP depletion on mood in depressed patients. METHOD: 87 depressed patients (DSM-III-R criteria) who had not received at least 40 days of a tricyclic antidepressant (TCA) or monoamine oxidase inhibitor (MAOI) and who had not been exposed to tryptophan depletion (TD) and control testing) for the TRP depletion were consequences. On one test the diet and drug were supplemented with 1 TRP control and on the other test the diet and drug treatment were paired. Behavior assessments (Hamilton Depression Scale (HDS), plasma and plasma TRP levels) were obtained prior to and during testing. RESULTS: Total and free TRP decreased to 90 % after the TRP-depletion (TD). 35 % of 47 symptomatic, drug-free depressed patients were unchanged the day the TD, but became clinically depressed (40% mean decrease in HDS, 95 point decrease in total HDS score) the day after the TD. 80 % of 40 antidepressant-remitted depressed patients relapsed (300% mean HDS increase) the day of the TD, which returned to remitted state the day after. While 40% of mianserin, 40% of fluoxetine treated patients relapsed, only 20% of desipramine-taxed patients relapsed. Implications: Rapid depletion of plasma TRP transiently reverses antidepressant response in most remitted depressed patients suggesting that the antidepressant effects of some of these drugs may be more dependent than that of others. Clinical characteristics and ultimate treatment response of symptom, drug free depressed patients in relation to the behavioral response to TD will be presented.

377.9 MEPACINE BLOCKS DESIPRIPINE INDUCED BETA ADRENEOCEPTOR DOWNREGULATION IN C6 GLIOMA CELLS. H.L. Manji, G. Chen, J. Bitora*, S. Gouovsky, and W. Z. Potter*, Dept. Clin. Pharmacol., Clin. Neurosc. Branch, NIMH, and Lab of Biochemical Chemistry, NIDDK, NIH, Bethesda, MD 20892. Chronic treatment with a number of antidepressants (AD) results in a downregulation/desensitization of beta cortical beta adrenoceptors (β-AR). This effect has generally led to an elevation of synaptic norepinephrine, the recent demonstration of similar β-AR alterations in C6 glioma cells and human fibroblasts following treatment with antidepressants (AD) has also implicated direct post-synaptic mechanism(s). Mepacine (MDP), a phospholipase A2 (PLA2) inhibitor, has been shown to attenuate agonist-induced β-AR downregulation in C6 glioma cells and rat hypothalamic tissues. In order to investigate the possible role of PLA2 in AD-induced β-AR downregulation, C6 cells were incubated with DMI (10μM) alone, or in combination with MDP (10μM) for 5 days. DMI resulted in a 25% decrease in the density of β-ARs, and a similar reduction in isoproterenol (but not forskolin) stimulated cyclic AMP accumulation in intact C6 cells. MDP alone had no effect on β-ARs or β-ARs sensitivity but markedly attenuated both the DMI induced β-AR downregulation and desensitization; these results suggest that arachidonic acid and/or its metabolites may be involved in DMI's effects. Preliminary results suggest that protein kinase C (PKC) is not involved in DMI's effects in C6 glioma cells.

377.11 NEUROENDOCRINE EFFECTS OF TANDOSPIRON (SM-5397) IN HEALTHY MALE SUBJECTS, C. T. FISCHETTE, P. L. DELGADO, J. S. BIRCH, J. H. KRISTAL, G. R. HENINGER, D. S. CHARNES, Dept. of Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06508 and Pfizer Pharmaceuticals, Inc., NY, NY 10017. Tandospiron is a selective 5-HT1 partial agonist, currently being studied for its efficacy in depression. This study evaluated the effects of tandospiron in feeding healthy subjects. Subjects received single doses of either placebo, 30, 40 or 50mg tandospiron at weekly intervals. At each testing session beginning at approximately 8:00 AM, blood was collected via an indwelling catheter at -20, -5, 30, 60, 90, 120, 150 and 240 minutes after oral drug administration for plasma levels of growth hormone (GH), prolactin and cortisol. In preliminary data (N=7) peak minus baseline GH concentrations were (mean ± SD): 0 ± 2.2ng/ml after placebo and 7.3 ± 7.1ng/ml (p<0.05), 9.2 ± 7.6ng/ml (p=0.05) and 7.7 ± 8.4ng/ml (p=0.09) after 30, 40 and 50mg tandospiron, respectively. Cortisol levels peaked at 72-s after tandospiron and showed no other significant changes. Tandospiron exhibited rapid absorption and metabolism to 1-phenyl piperazine (1-PP), a common metabolite of this class of drugs which exhibits alpha2 amnagonist activity in animal studies. 1-PP levels were much higher than the parent compound. This linear, dose related increase in 1-PP levels suggests differential regulation of these hormones, with serotoninergic and/or alpha2 noradrenergic receptor systems.

377.12 CONPARISION OF THE EFFECTS OF MIANSIR JN ANTIMONITORS AND METABOLITES ON A BEHERRAL SCHEN FOR ANTIDEPRESSANT ACTIVITY. C.J. Marek, T.H. Hand and L.S. Setlen, Dept. Pharm/Physiol.Sci., Univ. of Chicago, Chicago, IL 60637. The behavioral effects of racemic mianserin, its (+) and (-) enantiomers, and its metabolites dimethyl-mianserin and 8-hydroxymianserin were evaluated on the differential-reinforcement-of-low-rate (DRL)-72-s schedule, an operant behavioral screen known to be sensitive to and specific for the antidepressant properties of drugs. Racemic mianserin and the antidepressant-like effect (increased reinforcement rate, decreased response rate) at 5 and 10 mg/kg. The mianserin enantiomers showed the antidepressant-like effect at doses up to 3 mg/kg. This conclusion is not supported by the (+) mianserin-2.5 mg/kg. The mianserin metabolites showed no clear dose-related effects at doses up to 3 mg/kg, and the antidepressant-like effect of mianserin was due to the activity of the parent compound rather than to its enantiomers, and that the antidepressant-like effects of mianserin on DRL behavior are mediated through antagonistic action at the 5-HT1 receptor. This work was supported by PHS MH-11193 and RSA-10565 (L. Setlen).
377.13

down-regulation of 5-HT2 receptors and behavioral responses following chronic treatment with antidepressant drugs. J. Lucki and S. Winder.

377.14

chronic administration of phentolamine and N-acetylphepinephrine causes elevation of whole brain amine neurotransmitter levels and 5HT receptors.

377.15


377.16


377.17


377.18

377.19
ALTERED FENFLURAMINE-STIMULATED PROLACTIN RELEASE DURING TREATMENT OF OBSESSIVE COMPULSIVE PATIENTS. W.A. Hewlett, Dept. of Psychiatry, Stanford University School of Medicine, Stanford, CA 94305.
OCD is a psychiatric disorder which may involve alterations in serotonin functioning. In order to assess serotonergic functioning in OCD patients fenfluramine-stimulated prolactin release was measured in both a medication free condition and during treatment with either clomipramine, clonazepam, clomipramine, or diphencypram. Two baseline prolactin samples were drawn 30 minutes apart prior to administration of a 60 mg oral dose of fenfluramine. Fenfluramine-stimulated prolactin samples were than drawn hourly for the next five hours. Treatment with clomipramine significantly elevated baseline prolactin levels as compared to the untreated condition. Female patients showed significantly greater elevations in fenfluramine-stimulated prolactin release in both treated and untreated conditions. Fenfluramine-stimulated prolactin release was significantly greater under conditions of clomipramine and clomipramine treatment than that seen during diphencypram treatment. These results are consistent with altered serotonergic functioning during serotonergic treatment of OCD.

377.21
These double-blind placebo-controlled studies examined the efficacy of adding lithium carbonate, which may augment serotonin (5-HT) function, to an ongoing treatment trial of fluvoxamine (50%) in patients with primary OCD who had failed to respond to FXV.METHODS:Study 1: 20 patients (4 impatients, 16 outpatients) with OCD (DSM-III-R) were randomized to 2 weeks of treatment with active (N=11) or placebo (N=9) lithium augmentation of ongoing FXV treatment. Study 2: An additional 10 outpatients with OCD were randomized to 4 weeks of treatment with active (N=5) or placebo (N=5) lithium augmentation of ongoing FXV treatment. Outcome was assessed with Y-BOCS scores before and after the addition of lithium to FXV. RESULTS:Study 1: Two of 11 (18%) met criteria for a meaningful clinical response (1 marked, 1 partial) to active lithium. No patients responded to placebo. Active lithium augmentation of FXV produced a statistically significant improvement in scores on the Y-BOCS (4.0+5.2, p<.005, paired t test, two-tailed). There was no significant change in severity of OCD symptoms in the placebo-lithium-treated group. There were significant between-group differences as measured by the Y-BOCS (p=.04, student’s t test). An analysis of all patients treated subsequently with 3-5 weeks of open active lithium augmentation showed a statistically significant improvement in Y-BOCS scores (-3.1+2.5, p<.005, 51/61% of patients showed a qualitative response (1 marked, 4 partial). Study 2: No patients demonstrated a response during the 4-week controlled trial of lithium augmentation of FXV. In addition, there was no significant change in scores on the Y-BOCS. Conclusion: Administration of lithium to patients receiving 4 weeks of open active lithium augmentation of FXV (N=20), no significant change in Y-BOCS scores was found (-3.1+5.2, n.s.). Only 4/20 (20%) of these patients showed a response (1 marked, 3 partial). This double-blind placebo-controlled trials suggest that lithium augmentation is not an effective strategy in most OCD patients. The different response to this treatment strategy between patients with OCD and depression suggests that pathophysiological differences may exist between these two disorders.

LEARNING AND MEMORY: PHYSIOLOGY V

378.1
ORBICULARIS OCULI AND EXTRAOCULAR MUSCLE ACTIVITY DURING UNCONDITIONED AND CONDITIONED EYEBLINKS IN THE RABBIT. N.E. Berthier, J.W. Moore, Department of Psychology, University of Massachusetts, Amherst, MA 01003.
The activity of the retractor bulbi (RB), superior rectus (SR), and orbicularis oculi (OO) muscles were recorded in awake animals using chronically and acutely implanted teflon coated microwires. Eyelid and nictitating membrane positions were measured with potentiometers. All three muscles showed a two component response to trigeminal nerve stimulation; air puff and periodontal field stimulation. With periodontal field stimulation the latencies of the two responses were about 5 and 10 ms. Crosscorrelograms of EMG activity taken from conditioning trials showed less than 5 ms shift in the peak correlations. Examination of individual conditioning trials showed that the time course of EMG activity in the RB, SR, and OO muscles was highly correlated. Two differences in EMG activity were seen between muscles: (1) The OO showed tonic activity while the RB and SR did not. (2) The SR showed small EMG responses relative to the RB and OO. Given the high correlation between the activity of extraocular and OO activity we conclude that muscle activity during blinks is the result of a single premotor system. (Supported by AFOSR 89-0391 and BNS 88-10624).

378.2
To investigate the role of brain DNA synthesis in non associative learning, adult male albino rats of a random-bred Sprague-Dawley stock (NRE), and of the Naples High (NHE) and Low-Excitability (NEL) strains, selectively bred for divergent activity in a novel environment (Sadile et al., Soc.Neurosci. 9:643, 1983) were used. Three groups of rats, non-injected and injected with 100 mcg of triiodothyronine 15 min before test trial 1 or 2. Brain DNA synthesis was measured in ex-vivo homogenates of neocortex, hippocampus, neostriata, midbrain, hypothalamus and cerebellum by the incorporation of 3H-thymidine into DNA after a 30min pulse. Both NLE/NHE rats had a higher basal DNA synthesis in the hippocampus and neostriata. Upon test trial 1, but not upon re-test, there was a significant decrease in DNA synthesis in the neocortex and hippocampus of both strains, and in the hypothalamus, midbrain and cerebellum of NLE only. The decrease was lower in NHE than in NLE rats. The results support the proposed involvement of brain DNA in information processing and storage.
378.3

LEARNING AND MEMORY: PHYSIOLOGY V

INNEX FROM NICOTINE-INDUCED CONDITIONED GOO AVERSIO IN MICE. N.E. Kinney, Dept. of Psychology, SR Missouri State Univ., Cape Girardeau, MO 63701.

Mice (Mus musculus) demonstrated dose-dependent conditioned food aversion (CFA) when normal food (almond) consumption was paired with a subcutaneous injection of nicotine tartrate (0.2 - 1.6 mg/kg). Controls showed no aversion (but rather a significant increase in consummatory behavior) at 0.2 mg/kg, and complete aversion at 1.6 mg/kg. This contrasts with reported aversion to nicotine at 0.1 mg/kg in rats. Immunity to nicotine-induced CFA was demonstrated by bilaterally olfactory nerve-sectioned mice on day 7 post-surgery (while anesthetized). This potentiation was lost by day 15 post-surgery.

The novel food effect shown by controls was not observed in nerve-sectioned mice. These findings are in agreement with earlier work using tilted-rotary motion to produce malaise. The loss of CFA in anosmic animals (and its appearance following olfactory nerve regeneration) for both notion-induced and nicotine-induced malaise, strengthens support for a dominant role of olfaction in the formation of conditioned flavor aversions.

378.4

PLASTICITY IN THE REINFORCEMENT SYSTEM OF INFANT RATS. R.M. Sullivan and D.A. Wilson. Developmental Psychology Laboratory, Dept. Psychology, University of Oklahoma, Norman, OK.

Newborn rat pups learned to associate oral stimulation with tactile stimulation which mimics stimulation during maternal care. This learned behavioral preference is associated with a modified olfactory bulb neural response to odor. Odor-stroking in pups after PN9-10, however, do not produce these learned behavioral responses, suggesting a sensitive period in early olfactory development. The present study examined the nature of this age sensitivity.

Pups were trained on PN5, PN12 or PN20 in an olfactory conditioning paradigm using 3-D DEOXYGLUCOSE IN MICE. F. Gonzalez-Lima S.M. Scheich, and H. McLaughlin, Department of Anatomy, Texas A&M Univ., College Station, TX 77843; Inst 2001, Tech Univ, Darmstadt, W Germany.

The 2-DG method was used to image associative activity in twelve split-brain rats and gerbils. Training started 2 weeks after surgery and consisted of unilateral presentations of sound and foot shock in paired (one side) and unpaired (other side) sessions. The next day, subjects were tested for conditioned suppression of drinking, injected with 2-DG, and stimulated binaurally with sound. Differences in 2-DG uptake between the two sides of the brain were quantified. The conditioned hemisphere (side contralateral to paired stimuli) showed striking enhancements of uptake in forebrain structures, including hippocampus, subiculum, accumbens, entorhinal and perirhinal cortex, amygdala, and fundus striati. The largest increase (70%) was in the CA2 region of the hippocampus. The findings revealed for the first time a direct axis, within subjects, comparison of the cerebral representation of auditory memory. (Supported by NIMH grant RO1-MH43335.

378.6

MAPPING OF AUDITORY MEMORY WITH 2-DEOXYGLUCOSE: LATERALIZED CONDITIONING EFFECTS IN SPLI-RAIN RATS AND GERBILS. F. Gonzalez-Lima, S.M. Scheich, and H. McLaughlin, Department of Anatomy, Texas A&M Univ., College Station, TX 77843; Inst 2001, Tech Univ, Darmstadt, W Germany.

The 2-DG method was used to image associative activity in twelve split-brain rats and gerbils. Training started 2 weeks after surgery and consisted of unilateral presentations of sound and foot shock in paired (one side) and unpaired (other side) sessions. The next day, subjects were tested for conditioned suppression of drinking, injected with 2-DG, and stimulated binaurally with sound. Differences in 2-DG uptake between the two sides of the brain were quantified. The conditioned hemisphere (side contralateral to paired stimuli) showed striking enhancements of uptake in forebrain structures, including hippocampus, subiculum, accumbens, entorhinal and perirhinal cortex, amygdala, and fundus striati. The largest increase (70%) was in the CA2 region of the hippocampus. The findings revealed for the first time a direct axis, within subjects, comparison of the cerebral representation of auditory memory. (Supported by NIMH grant RO1-MH43335.

378.7

NEURAL SUBSTRATES FOR DISCRIMINATIVE OPERANT CONDITIONING VISUALIZED USING 2-DEOXYGLUCOSE AUTORADIOGRAPHY. M. Garrens, F. Gonzalez-Lima and F.J. Helmslebet. Dept Med Anat, Texas A&M Univ, College Station, TX 77843; Univ Valladolid, Spain.

2-DG techniques applied to rats trained in four groups were used to map the neural representation of the behavioral components involved in discriminative operant conditioning. 1) Rats trained to bar press for food during a sound discriminative operant conditioning (sound -> operant CR -> reward); 2) Rats without bars yoked to those in Group 1 (sound -> reward); 3) Rats trained to bar press for food, independently of sound, nondiscriminative operant conditioning (random sound, operant CR -> reward); 4) Rats without bars yoked to those in Group 3 (random sound and reward). Group 1 rats (disc) compared to 3 (nondisc) showed 2-DG changes in acumbens, ventral tegmental area and the parietal thalamic nuclei. Structures with the same 2-DG uptake in the operant groups (1=3) but different than the nonoperant groups (2.4) included frontal cortex, parietal and reticular thalamic nuclei, medial geniculate and cerebellar vermis. Common changes in the conditioned groups (1=2=3) but different than the random sample (4) included the cerebellar dentate, posterior parietal cortex, dorsal cochlear nucleus, and cerebellar floculus. These changes are the first demonstration of brain metabolic alterations related to a learned discriminative response to sound. (Supported by NIMH grant RO1-MH 43335.)

378.8

PHYSIOLOGICAL PROPERTIES OF MEDIAL SEPTAL NEURONS (MSN) IN UNANESTHETIZED RATS. J.E. Sweeney, M.H. Bassant* and Y. Lamour, INSERM U 161, 75014 Paris France.

Rhythmically bursting neurons (RBN) of the medial septum may trigger one type of hippocampal theta rhythm that correlates to behavior in the rat. Since the activity of RBNs varies with different anesthetics, we have chosen to characterize their properties in unanesthetized rats habituated to a painless restraint.

In 353 MSNs (recorded from 12 rats, 59 neurons (17%) exhibited a rhythmic bursting activity. This percentage is clearly different from the 45% of bursting MSNs recorded in urethane-anesthetized rats. The bursting activity was also of a significantly higher frequency than with urethane (5.8 ± 0.1 vs 8.0 ± 0.1 Hz). A burst consisted of 4-9 spikes/sec. The burst frequency of bursting neurons was recorded during EEG arousal (with a theta). Nevertheless, 2 bursting neurons were recorded in "drowsy" states (in the absence of a theta) and 10 during paradoxical sleep (with a frequency of 62 ± 62 bursts/sec). Tactile or electrical stimulation of the reticular formation induced EEG arousal and rhythmicity in 13 of 58 previously non-bursting neurons that had a high (>1 spikes/sec) spontaneous activity. Clearly, properties of rhythmically bursting neurons differ between unanesthetized and urethane-treateated rats. These results also suggest that there may be discrete pools of MSN—a small number that maintains rhythmicity regardless of the state of arousal—actively recruiting cells. High frequency firing cells that can be recruited by an arousing stimulus to fire rhythmically.

This work was supported by a NATO postdoctoral fellowship to JES and a grant from Bayer-Pharma France.
Reversibility in the most septal temporarily disrupts spatially selective discharge of hippocampal hilar/CAL and (not CA1) cells, reduces movement-sensitivity of stratum granulosum (SG) units, and produces a spatial working and/or long-term memory in rats (Mizumori et al., J. Neurosci., 1989). Recently, we examined the possibility that septal inactivation produced these effects by functionally isolating hippocampus from its primary input (entorhinal cortex) and/or output (subicular) structures.

Spontaneous single unit activity was monitored in hippocampus (CA1 & 2; n=16; FD: n=16), dorsal subiculum (n=20), and medial entorhinal cortex (n=13) before, during, and after septal inactivation in 10 Nembutal-anesthetized F-344 rats. Baseline activity was recorded for 10 min. Lido (0.5 μl; 2% solution) was injected into the medial septal area, then unit activity was monitored for at least an additional 20 min. Similar to previous results, few (6%) CA1 complex spike cells and 50% of SG and hilar/CAL cells showed altered discharge patterns following lidocaine treatment. A small percentage of subiculum (10%) and entorhinal (15%) cells responded by either increasing or decreasing firing. Ongoing experiments with freely behaving animals thus far confirm this pattern of responses. These data suggest that septal inactivation-induced alterations in subcortical, rather than entorhinal, afferents underlies the hippocampal unit effects. Furthermore, the behavioral impairment described earlier may have resulted from a disruption of hippocampal, and not subicular, unit activity.

Supported by BRS5 Grant 507 R017092.

378.11

Functional restoration in the cholinergic system with age may be partly responsible for age-related memory deficits. Therefore, activation of cholinergic neurons in the basal forebrain may alleviate these memory impairments. In the present study, naïve young (4 mo), scopolamine-treated young (4 mo), or naïve old (24 mo) rats were microinfused into the medial septal area (MSA) with cholinergic receptor agonists (carbachol, oxotremorine), a GABA antagonist (bicuculline), or saline. Working memory, as assessed by choice accuracy in a spatial alternation task, was tested before and after infusion, then compared to baseline performance during saline and non-infusion trials. Preliminary data indicate that cholinergic agonists infused directly into MSA can partially reverse scopolamine-induced deficits in young rats and may also improve performance in the aged rats in this task. These data suggest that cholinergic drugs may act directly on basal forebrain cholinergic neurons for their mnemonic effects. Hippocampal EEG recordings before and after microinfusion are currently being analyzed for electrophysiological correlates of the behavioral data. Supported by NRSA # NS8616.

378.13

Hippocampal caspase-3 is elevated in the rat hippocampus in the absence of neuronal death in response to excitotoxic insults. The increase is anotomically localized to neurons previously shown to be critically involved in the storage of a classically conditioned response which has been shown to undergo specific biochemical, electrophysiological, and morphological modifications. The present study confirms and extends these previous results while implicating anatomically-localized increases in PKC to learning-specific molecular processes.

The circumsphagial ganglion of animals in three treatments (Paired, Random and Naive) were infused with 3H-PDBu and then processed for histology and subsequent computerized image analysis. Paired, but not Random or Naive, animals showed a significant increase in 3H-PDBu binding in the medial and intermediate B photoreceptors and in cells of the optic ganglion (17%, p<.01, a=n per group, planned orthogonal contrast). This increased binding of 3H-PDBu in Hippocampal represents to our knowledge the first conclusive evidence for neuronal specific changes in the distribution of this regulatory enzyme (Olds et al Science, 1989).

378.14
GABA-INDUCED PROTEIN KINASE ACTIVATION IS ENHANCED WHEN PAIRED WITH POST-SYNAPTIC DEPOLARIZATION.
L.D. Matzel and D.L. Alkon
Laboratory of Molecular and Cellular Neurobiology, NINDS-NIH

The characteristic inhibitory influence of vestibular hair cells on the B photoreceptors of the Hermisenda eye is mediated by a GABergic synapse. Hair cell impulses that accompany a Lombardi (random) as well as exogenous GABA (12.5 μM) application activate a primary C-type conductance that is accompanied by a decrease in membrane resistance. Activation of a second, slower K+ conductance, characterized by an increase in membrane resistance, is also observed 60-90 sec after GABA application. The K+ channel blocker NPPB (10 μM) was added to the extracellular bath, this increase in resistance was observed within 10 sec of GABA application, suggesting the slow conductance is normally masked by the faster C conductance. Moreover, the K+ channel blocker TEA (100 μM) was added to the bath, this markedly increased in resistance was dramatically enhanced. This latter effect was not observed if Ca ++ was removed from the extracellular bath or if the protein kinase inhibitor H7 (100 μM) was added to the bath, suggesting that the activity of a GABA-activated protein kinase was enhanced by depolarization-induced intracellular Ca ++ elevation in the post-synaptic neuron. These results suggest a mechanism underlying the temporal dependence of stimulus pairings in classical conditioning, and in particular, the biophysical changes in single neurons which accompany light-rotation pairings in Hermisenda.
378.1  
RESPONSE OF TEMPORAL LOBE NEURONS TO SOCIAL STIMULI IN MACACA ARCTOIDES. L.A. Brothers, B.D. King* and A.S. Ellis. UCLA/Seplulveda VA Medical Center, Sepulveda, CA 91343

Accurate evaluation of social signals is essential to group-living primates; however, neural processing of the full range of social stimuli remains little understood.

We used laser disks containing 50,400 frames of moving pictures and vocalizations of macaques recorded in natural settings to deliver 2s clips of a broad array of body parts, movements, expressive and social interactions. Recordings of single unit activity in the region of the right amygdala and neighboring cortical areas were obtained in an alert stump-tailed macaque.

Of 275 isolated units, 21 were selectively responsive to a subset of the presented stimuli. Of these, 2 responded to one or a few specific pictures only. The remaining responded to a heterogeneous subset of the stimuli or were selective for a single feature common to a number of stimuli. These results suggest that anterior temporal neaurons of the macaque brain participate in ensembling coding features of the social environment.

Supported by the Dept. of Veterans Affairs.

378.2  
SPATIAL TUNING IN MUSTACHE BAT AUDITORY NEURONS WITHIN THE CONTEXT OF ECHOLOCATION. Z.M. Fuzessery, Dept. of Psychology and Zoology, Univ. of Wyoming, Laramie, WY 82071

Echolocation in bats is a complex and highly specialized form of spatially tuned auditory perception. This study examines how spatial processing in the mustache bat might be optimized. It concerns a morphological and behavioral transformation that the mustache bat undergoes when echolocating. One finding is that intensity level is almost constant across the center of the sound field, from 40° to both sides of the mid-sagittal plane. This is not the case when only ear directionality is considered. Moreover, binaurally facilitated neurons that exhibit a broad spatial tuning when only ear directionality is considered show a sharper selectivity when evaluated within the context of echo-location. In addition to maintaining an almost constant intensity level across the center of the sound field, the mustache bat also "intensity compensates" (Henson et al., 1985) to maintain the echo at an absolute intensity level. These findings suggest that this echolocation system may be optimized to reduce the ubiquitous problem of differentiating stimulus quality and quantity.

NS-13276, NS-21286.

379.1  
NEUROETHOLOGY: MAMMALS, REPTILES, AMPHIBIANS

379.2  

Encoding of stimulus repetition rate and duration by the inferior colliculus neuron(IC) of the big brown bat, Eptesicus fuscus was studied by recording responses of each neuron to a wide range of repetition rates and durations at several stimulus intensities under free field stimulus conditions. A total of 277 IC neurons were recorded at depths between 147 and 2370 μm with latencies between 3.4 and 28.4 ms. They were tonotopically organized along the dorsoventral axis of the IC according to their best frequency(BF). While individual IC neurons varied their number of impulses but not their discharge pattern when stimulated with different repetition rates and durations, they generally discharged with a maximal number of impulses to a specific repetition rate (the best repetition rate) and duration (the best duration). According to the filtering properties for the stimulus repetition rate or duration, IC neurons can be classified as high-pass, low-pass, band-pass or semi-band-pass and multi-pass neurons. A wide range of repetition rate and duration best responses were identified for IC neurons but they were not correlated with either the BF or recording depths. Furthermore, the best repetition rates and durations of tonotopically organized IC neurons isolated within an area of the IC was not correlated with either the BF or recording depths. According to the correlation of their responses to stimulus duty cycle, IC neurons can be classified into two groups. Responses of one group correlated with the duty cycle thus enable the bat to avoid pulse-echo overlap during echolocation. Responses of the other group do not correlate with the duty cycle. These neurons likely enable the bat to encode the pulse emission rate and duration effectively during different phases of hunting. The effect of stimulus repetition rate and duration on the intensity rate function of IC neurons was examined. While the stimulus repetition rate might affect the dynamic range and overall profile of the intensity rate function curve, only little effect was observed with different stimulus durations.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
SPECTRAL SELECTIVITY OF DELAY-SENSITIVE NEURONS IN THE AUDITORY CORTEX OF AN FM BAT." P. Wong and H. Mackaya, Anatomy Dept., Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Spectro-temporal processing plays prominently in auditory perception. In the echolocating bat, frequency selective, delay-sensitive, cortical neurons process target-distance information in the time domain. The concurrent role of spectral processing in target-distance perception was further examined neurophysiologically. Delay-sensitive neurons were isolated by stimulation with FM-FM sound pairs at frequency excitations mimicking natural bat echolocation sounds (pulse) and echoes. The pulse and echo FMs were each arbitrarily divided into four spectral quarters and the quarter used for delay-sensitivity was determined. Neurons typically required a spectral combination consisting of both the IV pulse-quarter and the III or IV echo-quarter. The essential echo component did not change when the rate of pulse-echo stimulation was increased. The preservation of this spectral combination for delay-sensitivity suggests that throughout echolocation FM bats employ specific parts of their pulse-echo spectra for perceiving target structure. (Supported by NIH grant DC00600).

CENTRAL MONOAMINES AND BEHAVIOR IN THE LIZARD, ANOLIS CAROLINENSIS R. Greenberg, G. W. Sumner, and K. H. Dagan. University of Tennessee, Knoxville, Graduate Program in Ethology; Stanford University Medical Center.

To clarify monoamine function in stereotyped behavior, the selective neurotoxin MPTP was used to deplete dopamine (DA) and norepinephrine (NE) in a model species, the lizard, Anolis carolinensis. Following the intraperitoneal injection of MPTP, animals exhibited spontaneous auditory sensitivity normally not displayed under drug or normal or slightly facilitated aggression and reproductive body behavior. Body color change, an index of altered automatic sensitivity and responsive behaviors, was seen. Earlier work (Font et al. 1987) revealed MPTP-induced central neuropathies. Immunohistochemistry was employed to discern specific central catecholaminergic systems thereby affected. DA and NE immunoreactive perikarya were found in several sites in and near the periventricular hypothalamic nuclei, ventral tegmen tal nucleus, substantia nigra, locus ceruleus, nucleus of the solitary tract, and raphe; while immunoreactive NE cells were found in one periventricular hypothalamic site and in the raphe nucleus.

Estimates of quantitative change in catecholamine levels obtained with high pressure liquid chromatography revealed that 100 h after MPTP injection, DA and NE levels were 26% and 7% of control, respectively, while 5-HT was elevated to 200%.


Studies of the effects of activating the lateral line system on the feeding behavior. We used two methods to block the lateral line system: 1) the exposure to collicidal solution [Co treatment] and 2) the transection of cranial nerves supplying the ear region and lateral line system (roa and ram). We examined the effects of cutting the lateral line system on the feeding behavior. We used two methods to block the lateral line system: 1) the exposure to collicidal solution (Co treatment) and 2) the transection of cranial nerves supplying the ear region and lateral line system (roa and ram). We examined the effects of cutting the lateral line system on the feeding behavior. The feeding behavior was elicited by the water movement produced by the vibrating sphere (frequency: 2-50Hz, amplitude: 1-4mm). The vibrational stimuli were as an visual stimulus, suggesting that sensory system other than vision was involved in the feeding behavior. In the blocking experiment of lateral line system, the feeding response disappeared.


While phasic neurons are considered to be essential for encoding the time of a sensory event, other specific functions these neurons serve in acoustic information processing have not been fully elucidated. Given that various physiological/functional features (e.g., rate, duration, and rate of amplitude modulation (AM) of frog calls serve as essential cues for call discrimination, we sought to identify specific features of the phasic response that are likely to be involved in the coding of temporal patterns of complex sounds. With this as a working hypothesis, we have systematically recorded single unit activity at various points in the auditory system of the frog Rana pipiens and Rana pipiens in order to characterize the role of phasic neurons (relative to tonic neurons) in the encoding of such behaviorally significant temporal sound parameters.

With respect to the processing of signal rise-time duration and time, duration, phase neurons in the auditory brainstem (e.g., the external, and torus semicircularis) develop temporal selectivities typically not manifested by tonic neurons. For example, many neurons were found to be "tuned" to a narrow range of stimulus rise times, and rate of AM. Thus, in addition to coding for the arrival time of an acoustic stimulus, phasic neurons also act as "temporal filters" with respect to rise-time duration, signal duration, and rate of AM. This suggests that the temporal selectivity of phasic neurons for various temporal parameters may be involved in processing sounds in the acoustic communicative behavior of frogs and perhaps other species.
379.11 THE INFLUENCE OF SOUND DIRECTION ON PROCESSING OF COMPLEX SOUNDS BY MIDBRAIN AUDITORY NEURONS. D. M. Golder, C. L. Condon1, and A. S. Feng. Dept. of Physiology and Biophysics, and 1Neuroscience Program, University of Illinois, Urbana, IL 61801.

Head and body orientations are important for acoustic communication in complex environments. Certain animals change their body orientations to improve sound localization, and/or to aid in the detection of complex sounds. In this study, we measured the influence of sound direction on responses of various species of birds and mammals. Our results revealed that optimal orientations were not always the same for different species. These observations suggest that optimal orientations are related to the species' behavioral needs.

We have recorded from single neurons in the auditory midbrain of several species of birds and mammals. The recordings were made using glass microelectrodes inserted into the inferior colliculus or the area tegmentalis. The sound stimuli were presented in a variety of directions, and the responses of the neurons to these stimuli were recorded. Our results suggest that the optimal orientations for different species are related to their natural habitats and behaviors.

379.12 COMPARISON OF TIME-LOCKED RESPONSES OF FROG'S CENTRAL AUDITORY NEURONS TO SINU-SODIAL-AMPLITUDE-MODULATED SIGNALS WITH PHASE-LOCKED RESPONSES TO SINUSODIAL CARRIERS. W. Y. Lee, K. H. Wimbler. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

Single unit recordings were made from the frog's dorsal medullary nucleus (DMN), a homolog of cochlear nucleus, to determine whether or not the phase-locked response component to sinusoidal stimuli can be used to predict the time-locked response component to sinusoidal-amplitude-modulated (SAM) signals, and vice versa. For each cell, we first determined the best carrier frequency (BF) and the frequency tuning curve (FTC) of the unit. We then quantitatively analyzed the units responses to: (1) pure tone stimuli with carrier frequencies corresponding to the unit's FTC at 10 db above the respective thresholds of excitation, and (2) SAM stimuli at various modulation frequencies (ranging from 5 Hz to half of the BF or 1000 Hz, whichever was less) using the BF as the carrier at 10 dB above the excitation threshold at BF. The synchronization coefficient (SC) was calculated from the PSTH of the cell responses obtained from each of these responses using the formula given by Goldberg and Brown (1963). The SCs from time-locked and phase-locked responses at comparable frequencies (modulating vs carrier frequency) were compared.

We found that for mid- to high- frequency stimuli (BF=700-650 Hz), SAMs for SCs for SAM stimuli were typically lower than SCs derived from phase locked responses to sinusoidal carriers of comparable frequencies. For low-frequency sensitive stimuli (BF=100-650 Hz), the SCs derived for the two types of stimuli were comparable. (Research supported by NSF grant 88-09480.)

379.13 WITHIN AND BETWEEN POPULATION VARIATION IN THE ACOUSTIC COMMUNICATION SYSTEM OF CRICKET FROGS. A. C. Keddy-Hector,*, W. Wilczynski, and N. J. Ryan. Depts. of Psychology and Zoology, Univ. of Texas, Austin, TX 78712.

We compared variation in basilar papilla (BP) tuning and call dominant frequency in populations of Acris crepitans from Waverley (NB) and Stonalg Ranch (SR), Texas. The best carrier frequency (BF) and upper frequency limits of BF were determined for each of the 28 males examined in both populations. The females were collected from the same population as the males. The best carrier frequencies of females were significantly lower than those of males and lower than the dominant frequency of male calls. An inverse relationship existed between body size and BF tuning in both populations (SB male = r=-0.57; BF male = r=-0.62, female = r=-0.64). Analysis of covariance showed that body size differences do not account for the sex difference in BF tuning (BF male = 5.65, females = 5.10; BF male = 5.65, females = 5.10). The slopes of the female tuning curves were always lower than those of males.

These results suggest that in cricket frogs interpopulational differences in vocal and auditory traits result from concomitant shifts in the intraspecifical allometry of these traits with body size. (Supported by NIH 73310, NRH 88387 and NSF BNS 8606289.)

379.14 SEXUALLY DIMORPHIC LARYNGEAL MORPHOLOGY IN CRICKET FROGS (ACRIS CREPITANS). B. E. McCayland, W. Wilczynski and M. J. Ryan. Depts. of Psychology and Zoology, University of Texas, Austin, TX 78712.

The species Acris crepitans was studied in the field and in the laboratory. Male larynges are significantly larger than females, but differences exist among the species in the relative development of the female larynx. The male larynx is sexually dimorphic and varies with the presence or absence of vocalizations in the male's behavioral repertoire. Because female call acoustic (Acris crepitans) are completely silent, we predict extensive quantitative and/or qualitative laryngeal differences between the sexes. In this study we used morphometric techniques to explore the differences between the anatomy of male and female larynges. Although the body size [snout-vent length: male (nm=12) 33.23 mm, female (n=12) 23.24 mm] and head widths of the males (.79 mm) and females (.78 mm) were not significantly different, the volume of each male (M) laryngeal component was much larger than the corresponding feature of the female (F) larynx (coordinates takeoff: M = 68, F = 0.06; vocal cord: M = 0.48, F = 0.004; CONDUCTOR MUSCLE: M = 64, F = 0.04; dilator muscle: M = 182, F = 0.023; all volumes mm3). Female vocal cords were subcutaneous and did not stretch across the laryngeal lumen which may have rendered them non-functional. Within each sex, the volumes of all the laryngeal features are positively correlated with body size. However, the slopes describing the male relationships are, in all cases, much steeper than the females'. Additionally, within each sex, the size relationships among the laryngeal components appear to be the same. In summary, although male larynges are considerably larger, the anatomical components of male and female larynges maintain the same allometric relationship within the sex. Not only the size, but also the lack of vocal cord development may prevent females from vocalizing. (Research supported by NSF BNS 8606289.)

379.15 ACOUSTICALLY SUBOPTIMAL MAL SPACE IN NYLIA CINEREA CHORDOES. K. H. Fox, W. Wilczynski, and W. M. Ryan. Dept. of Psychology, University of Texas, Austin, TX 78712.

Previous computer models of frog chorus acoustics have revealed that males can maximize per-capita mating call active space by utilizing "optimal" neighbor spacing (Fox and Wilczynski, 1985, SN Abstr. 101). Here, we examine whether local green treefrog (Nydia cinerea) males utilize this strategy. This species has two interindividual distances (ID) dependent on threshold distance (TD -- the distance at which a female can just detect a male's call), call duty cycle (DC -- the proportion of time the male produces sound), and chorus population. TD was determined by filtering population calls of female auditory, derived from multiunit auditory midbrain (torus semicircularis) responses to sinusoidal acoustic stimuli. DC was measured by playing tape recordings into an electronic timing apparatus. We find that TD = 377 + 181 m, DC = 129 + 0.03 s, and chorus population was typically ca. 10. 20. Therefore, computer models predict an optimal ID, 8.59 ± 0.88, is much lower than optimal. Such a large optimal ID must be impractical for N. cinerea because the resulting chorus would be too diffuse for females to traverse; therefore, "optimal spacing" would have little biological relevance. It now seems likely that active space maximization through use of optimal ID may be feasible only for species with lesser TD values, such as N. cinerea (Fox, 1986, SN Abstr. 148). (Supported by NSF BNS 8606289.)
380.1 TESTOSTERONE IMPLANTS RESTORE STEROID-SENSITIVE \( \text{HORMONAL} \) \( \text{RESTORES} \) \( \text{ANDROSTENEDIONE} \) IN CASTRATED RATS. (J. De Vries and R.M. Buja), Prog. in Neurosci. and Behav., Univ. of Mass., Amherst, and Netherlands Inst. for Brain Res., Amsterdam.

Vasopressin-immunoreactive (AVP-IR) projections of the bed nucleus of the stria terminals (BST) and the medial amygdaloid nucleus (MA) are steroid-sensitive. Gonadectomy wipes out AVP staining while subsequent testosterone treatment restores staining. However, when temperature of the hypothalamus is lowered in and around the BST and MA could restore this staining in long-term castrated rats. Implants in the BST of long-term castrated rats restored most of the innervation at the side of the implant, but there were differences depending on where the implants were placed. For example, implants in medial regions of the BST restored most of the bilateral fiber staining in the lateral septum but not the staining in the lateral habenular nucleus. Implants in lateral regions of the BST restored some fiber staining in the lateral habenular nucleus. Most fiber staining in the lateral habenular nucleus was restored, however, by implants in the MA. Since implanting testosterone between the BST and MA did not restore any fiber staining, the steroids acted probably locally in both nuclei. The differences in the effects of the implants might be directly related to differences in projections of subgroups of AVP cells in the BST and MA. Steroid implants may restore AVP fiber staining only in areas that receive direct AVP projections from the site of the implant. Alternatively, steroid implants might have stimulated other steroid-sensitive neuronal systems that, in turn, might have activated steroid-sensitive AVP neurons transiently. This would explain why implants in the MA restored much of the AVP innervation of the lateral septum, even though the MA projections to the lateral septum are much less dense than those of the BST.

380.3 GABA IN THE MIDBRAIN CENTRAL GRAY FACILITATES LORDOSIS IN THE FEMALE RAT: M.M. McCarthy, D.W. Pfaff and S. Schwartz-Giblin, Rockefeller University, New York, NY 10021

The inhibitory neurotransmitter GABA has been implicated in the control of female reproductive behavior. Microinjection of the GABA-A agonist, muscimol, into POA inhibits lordosis whereas muscimol into medial hypothalamic nuclei (McCarthy et al., Brain Res. 507: 229-236)

Using a 2-chamber apparatus in which a sexually active male is restricted to one chamber and a sexually inactive male to the other, behavioral parameters of lordosis were measured during 10 min tests. After pre-testing, steroid-primed females were infused with GABAergetic drugs via bilateral cannulae (0.25μg/ml) on the midbrain central gray (MCG) in estrous (E) or proestrous (P) females with high lordosis quotients during pre-tests, the GABA-A antagonist, bicuculline, significantly reduced lordosis and proceptive behaviors by 5 min at a dose of 30 ng but not 10 ng (p<.05; Wilcoxon Test). By hour one post-injection, lordosis quotients were equal to pre-test levels. Infusion of muscimol (50ng) did not affect behavior in E + primed females. Conversely, with low lordosis quotients and high avoidance index (# rejection/r # mounts) in E-primed females, muscimol (50ng) significantly increased lordosis quotient while decreasing avoidance index as compared to saline-infused controls at 5 and 60 min post-injection (p<.05; Mann-Whitney U).

In the estrous phase, lordosis was induced by castration and expression in the male chamber did not change after treatment with either drug. Infusion of the retrograde tracer FluoroGold (0.25μg) through each cannula after behavioral testing showed strong descending projections to MCG from the anterior hypothalamus, zona incerta, ventromedial nucleus of the hypothalamus and amygdala (central and medial nuclei). Ascending projections from lumbar cord included cells in laminae I, V, X and lateral spinal nucleus. Therefore, in addition to being facilitatory in the medial hypothalamus, GABA facilitates lordosis at sites which receive behaviorally relevant afferent inputs from forebrain as well as spinal cord.


Acetylcholine appears to be present throughout the songbird’s vocal control system. Vocal control nuclei (Vcn) concentrate steroids, altering neural functioning and acting behavior. Cholinergic neurons have been implicated in the control of song-mediated behaviors in other species and cholinergic enzyme activity in the Finch syrinx is hormone dependent. This study examined the effects of androstenedione (AE), the steroid that restores hormone dependent behavior in castrated finches, on cholinergic enzyme activity in the finch brain. Immature, castrated male, zebra finches received silastic implants of cholesterol or AE and were then paired with females in individual cages for one week prior to sacrifice. Choline acetyltransferase (CAT) activity in CA3 and CA1, the hippocampal CA3 and CA1 regions, respectively, were measured by immunoblotting of extracts of brain tissue. As expected, steroid treatment affected CAT activity in several of these nuclei. Interestingly, unlike the effects of steroid hormones on catecholamines, increased neurotransmitter levels and turnover in some areas but decreased levels in others, AE administration increased CAT activity in those brain areas in which it exerted an effect. (Supported by grants HD-15191 and MH-00591 to CFH.)

380.2 SPECIES AND SEX DIFFERENCES IN VASOPRESSIN PATHWAYS OF VOLES THAT SHOW DIFFERENT PATTERNS OF PARENTAL CARE. M. Bamshad, G.J. De Vries, M.A. Novak, Prog. of Neurosci. and Behav. and Dept. of Psychol., Univ. of Mass., Amherst, MA 01003

Prairie voles (Microtus ochrogaster) spend longer time in close contact with their young than meadow voles (Microtus pennsylvaniae). In addition, meadow voles males do not show any parental care towards the young, whereas prairie vole males do. In rats, body temperature of the mother is used to determine how long she nurses the young during each nesting bout (Leon M, Phys. Behav. 1978 21:759). Since vasopressin-immunoreactive (AVP-IR) projections of the bed nucleus of the stria terminals (BST) and medial amygdaloid nucleus (MA) have been linked to body temperature regulation (Pittman O, Brain Res. Bull. 1980 6:13-17) we studied whether there were differences in these projections that might help to determine why voles show these species-specific differences in parental care.

Brains of sexually inexperienced voles and voles that were six days postpartum were stained immunocytochemically for vasopressin. In both species, AVP-IR projections of the BST and MA were much denser in sexually inexperienced males than in females. This pattern was changed in parental animals. In females of both species, AVP-IR projections of the BST and MA were just as dense as in sexually inexperienced males. In meadow vole males, there were no differences between parental and inexperienced males, whereas in prairie vole males BST and MA projections showed less staining in parental animals. These findings suggest that, in females, BST and MA projections are involved in changes that take place when animals become parental. The sex difference in the direction of changes in parental prairie voles that become parental suggest that vasopressin might serve different functions related to parental behavior in both sexes.

380.4 GABAERIC CONTROL OF SEXUAL RECEPTIVITY IN THE FEMALE RAT. D.B. Masters, J.M. Fibiger and M.M. McCarthy, Institute of Animal Behavior, Rutgers University, Newark, N.J. 07102 U.S.A.

Recently, we reported that GABA agonists infusion into medial hypotalamus (mHYP) and preoptic area (POA) has facilitated sexual behavior, respectively (McCarthy et al., Brain Res 507, 1990). These data suggest that GABA differentially mediates receptive behavior. Therefore, we have studied the effect of GABA on lordosis in females. We have observed that GABA levels will be greater in mHYP than in POA in receptive (Rec) and opposite in non-receptive (Nrec) females along with concomitant changes in GABA receptor binding. mHYP (100 μg/kg) and i.c.v. (60 μg/kg) were administered 1 and 1 hr before these mHYP, POA & cortex (CTX) were dissected and frozen (in N2). Sections were homogenized in 1 ml 4% perchloric acid & 5 μl aliquots were analyzed for GABA (GABA-A receptor binding study done using receptor affinity of 0.90 kDa & high affinity receptor site was significantly" lower in POA of Rec vs Nrec & OXV rats, 1.1±.2 vs 1.5±.3 mg/mg respectively (α=7%; 0.05)). A parallel experiment was performed on the mHYP, POA (GABA-A receptor binding study done using receptor affinity of 0.90 kDa & high affinity receptor site was significantly" lower in POA of Rec vs Nrec & OXV rats, 1.1±.2 mg/mg respectively (α=7%; 0.05)). In conclusion, the data support our hypothesis that GABA plays a dual role in control of female mating behavior. Increased GABAergic activity in mHYP with a concurrent reduction in POA activity promotes receptivity, whereas the reverse inhibits this behavior. It is possible that tonic GABA levels and receptor dynamics in mHYP and POA are modulated by systemic steroids associated with the estrous cycle, regulating the natural onset and termination of receptive behavior in female rats. *ANOVA (p<0.05) was used.

1


Intracerebral administration of the acetylcholinesterase inhibitor, physostigmine, activates lordosis in intact, cycling female rats during proestrus, but not diestrus when estrogen levels are lowest (Menard et al., Brain Res 435, 1989). Therefore, cholinergic activation of sexual receptivity in intact female rats may be possible only when the female is pre-exposed to estrogen. To study this, cycling female rats received pulse injections (24 and 36 hr before behavior testing) of 0.2, 0.1, or 0.05 μg free estradiol or ethanol vehicle at 12 hr for Middiestrus. On the following day, females that were not sexually receptive (≤0.250) received intracranial injections of either saline or physostigmine (10 μg bilaterally) and then were tested for lordosis. Lordosis was increased 15 min (p<.0001) and 1 hr (p<.0003) after physostigmine injection on both days. The level of facilitation at 15 min (p<.0001) and 1 hr (p<.0212) increased with the levels of estradiol priming. No facilitation was evident with ethanol injection at either time. These results indicate that in intact female rats, cholinergic systems that regulate sexual behavior are dependent upon sufficient estrogen priming. Furthermore, estrogen levels above normal diestrus levels appear to be necessary for cholinergic regulation to occur.

USFRES grant ID-22235 and Louisiana Education Quality Support Fund 86-TU16(13)-111.
380.7

SEXUAL DIFFERENTIATION AND MONOAMINERGIC SYSTEMS: STEROID INFLUENCED BEHAVIORAL AND NEUROCHEMICAL CHANGES. M.A. Abdelnour*, D.W. Rollert, and N. Jaffer. Dept. of Psychology, Western Michigan University, Kalamazoo, MI 49008.


demonstrated that estradiol administered by the subcutaneous route during the critical period (Day 5 or Day 7) of embryonic development significantly alters CA levels and results in distinct sexual and vocal control nuclei. Similar changes in CA function were seen during the initial phases of the reproductive cycle. While hormone treatment affected both noradrenaline (NE) and dopamine (DA) function, the changes in NE were much more striking. We therefore decided to manipulate NE levels and examine the effects on courtship behavior.

Intact male quails were pretreated with zimeldine and then injected with N-(2-chloroethyl)-N-ethyl-2-bromo-benzylamine (DSP-4, 50ug/kg) or saline ip. Birds were observed for 15 minutes in pre- and post-drug pair tests. Vocal control was assayed with 24 hour song tests and the motor patterning of songs (cf. Turcotte, 1984). DSP-4 birds showed significant post-drug decreases in courtship behavior. This treatment increased the latency to sing and decreased the total number of song bouts, while the motor patterning of songs appeared unaffected. As expected, DSP-4 significantly depleted telencephalic (i.e., vocal control nuclei) NE while hypothalamic NE was not affected. Zimeldine pretreatment prevented significant changes in DA and 5-HT levels. These results suggest that the noradrenergic system is important in modulating courtship vocalizations in the zebra finch.

Supported by HD15191, MH05591 and PSC-CUNY 868237 to CFH, and MH09425 to SHR.

380.9

EFFERENT PROJECTIONS FROM THE VENTROLATERAL HYPOTHALAMUS IN FEMALE GUINEA PIGS. K.H. Nielsen and J.D. Blaustein, Neuroscience and Behavior Program and Psychology Department, University of Massachusetts, Amherst, MA 01003.

Implants of estradiol in the ventrolateral hypothalamus (VLH) are sufficient to prime ovariectomized guinea pigs to show progestrone-dependent lordosis. To determine the efferent projections from this area, Phaseolus vulgaris lectin (Pha-L) was applied stereotactically into the rostral VLH of ovariectomized guinea pigs. After a survival period of two weeks, the Pha-L was visualized immunocytochemically. The majority of Pha-L labelling was observed ipsilateral to the injection site, but occasional axons were labelled in the same contralateral structures. The following areas received the heaviest projections from the nucleus of the stria terminalis, medial preoptic area, anterior hypothalamus, tuber cinereum, ventromedial nucleus, dorsomedial and dorso-lateral hypothalamus, posterior hypothalamus, dorsal longitudinal fasciculus, midbrain central gray, and the rocess inferior colliculus. The three lateral areas also received the heaviest contralateral projections. Areas with fewer labelled terminals and axons included the diagonal band of Broca, lateral septum, medial and cortical amygdala, lateral preoptic area, paraventricular nucleus, arcuate nucleus, premammillary nucleus, dorsal tegmental area, and pontine central gray. Fibers with few or no terminals were observed in the median forebrain bundle, stria terminalis, and medial lemniscus. Many of these projections are similar to the efferents from the ventrolateral-ventromedial nucleus in rats, the area that is analogous to the VLH in guinea pigs.

Supported by NIH NS 19227 and RCDA NS 00970.

380.11

EFFECTS OF PYN LESIONS ON THE RESPONSIVENESS OF STEREOTACTICALLY INJURED RATS TO ESTRADIOL. P.C. Butera, R.M. Willard* and S.A. Raymond*, Dept. of Psychology, Niagara University, NY 14109.

This experiment tested the effects of estradiol on ingestive behaviors and body weight in female rats with bilateral lesions of the hypothalamic paraventricular nucleus (PVN). Thirty-two adult female rats received either bilateral or sham PVN lesions. All subjects were ovariectomized two weeks after the lesion. Two weeks following the surgery, two groups of rats were reversibly dejected by 2 ug of estradiol benzoate (EB) for 3 days and half were injected with the oil vehicle. After 10 days of recovery the hormone treatments were reversed. Behavioral and autonomic measures indicated that 9 females had bilateral PVN lesions and 4 had bilateral lesions of the dorsomedial hypothalamus (DMH). Eleven animals received sham lesions. There were no significant effects of oil injections on food intake, water intake, or body weight. EB significantly lowered water intake and body weight in all groups, however, food intake was suppressed in the DMH and Sham but not PVN-lesioned females. These findings indicate that the effects of estradiol on food intake are mediated by its actions in the PVN.

(Supported by NIH NS26020)

380.8


Previous studies have suggested that the Zebra finch may be involved in controlling courtship behaviors in male zebra finches. Hormone treatments which increased courtship behaviors in castrated males significantly altered CA levels and resulted in distinct sexual and vocal control nuclei. Similar changes in CA function were seen during the initial phases of the reproductive cycle. While hormone treatment affected both noradrenaline (NE) and dopamine (DA) function, the changes in NE were much more striking. We therefore decided to manipulate NE levels and examine the effects on courtship behavior.

Intact male quails were pretreated with zimeldine and then injected with N-(2-chloroethyl)-N-ethyl-2-bromo-benzylamine (DSP-4, 50ug/kg) or saline ip. Birds were observed for 15 minutes in pre- and post-drug pair tests. Vocal control was assayed with 24 hour song tests and the motor patterning of songs (cf. Turcotte, 1984). DSP-4 birds showed significant post-drug decreases in courtship behavior. This treatment increased the latency to sing and decreased the total number of song bouts, while the motor patterning of songs appeared unaffected. As expected, DSP-4 significantly depleted telencephalic (i.e., vocal control nuclei) NE while hypothalamic NE was not affected. Zimeldine pretreatment prevented significant changes in DA and 5-HT levels. These results suggest that the noradrenergic system is important in modulating courtship vocalizations in the zebra finch.

Supported by HD15191, MH05591 and PSC-CUNY 868237 to CFH, and MH09425 to SHR.

380.10

VENTROLATERAL HYPOTHALAMUS PROJECTIONS TO ESTROGEN RECEPTOR-IMMUNOREACTIVE SITES IN THE FEMALE GUINEA PIG BRAIN. J.C. Turcotte, K.H. Nielsen and J.D. Blaustein, Neuroscience and Behavior Program and Psychology Dept., University of Massachusetts, Amherst, MA 01003.

The ventrolateral hypothalamus (VLH), an area in which implantation of estradiol is sufficient to prime guinea pigs to display progestrone-facilitated sexual behavior, contains a large number of estrogen receptor-immunoreactive (ER-IR) cells. In rats, projections from the analogous ventrolateral-ventromedial nucleus, have been traced to a variety of brain sites which contain estrogen-concentrating cells. These projections may be part of a neural network which may be involved in the regulation of sexual behavior. In order to determine if efferents from the VLH project to ER-IR neurons, we immunohistochemically applied the antiserum to placebo phaseolus vulgaris Leucagglutinin (Pha-L) and visualized projections from this area with a double label immunocytochemical procedure. Pha-L injection sites were small and localized in sites containing a high concentration of ER-IR cells. VLH efferents were observed in many sites throughout the brain (Nielsen and Blaustein, 1990 Neurosci. abstr.), some of which also contain ER-IR neurons. The ER-IR containing areas which receive VLH projections include the medial preoptic area, the periventricular area and the midbrain central gray. Within these areas, some projections were found closely associated with ER-IR neurons suggestive of synaptic contacts. This finding supports the concept of a neural network of estrogen-sensitive elements within the VLH.

(Supported by NIH NS 19227 and NS 00970)

380.12

HYPOthalamic lesions facilitate the display of sexual behavior in the female golden hamster independently of photoperiod. A.S. Elliott* and A.A. Nunez, Dept. of Psych., Neuroscience Program, Michigan State University, East Lansing, MI 48824.

In order to further elucidate the role of the suprachiasmatic nucleus (SCN) in photoperiodic control of sociosexual behaviors, electrolytic lesions aimed at the SCN were made in female golden hamsters (Mesocricetus auratus) and the animals were then placed in either a long or short photoperiod. After ovarectomy, the estrogen-primed animals with lesions showed copulatory responses more frequently than those without lesions, regardless of photoperiod. No differences were detected when the estrogen treatment was supplemented with progesterone. Animals with lesions showed a marked increase in copulation frequency compared to controls, again regardless of photoperiod. Histology revealed that the actual placement of the lesions ranged from the prooptic area through the caudal aspects of the SCN. The lesions generally caused a decrease in damage in the anterior hypothalamus (AH), including the SCN, showed lesions much more frequently than those with damage solely in the prooptic area (POA). Control females with AH lesions showed less aggression than those with POA lesions. These data support the findings that the POA, AH and SCN may play a role in mediating the inhibitory action of the sexual-PGA on lordosis in female hamsters (Brain Res. 1980, 181:267; Physiol. Behav. 1982, 29:1131). Supported by grant BNS890876 from the NSF and by Biomedical Research Funds from M.S.U.
380.13
PREOPTIC AREA (POA) KNIFE CUTS AND KNIFE CUTS POSTERIOR TO THE VENTRAL TENTORIAL AREA (VTA) DISRUPT MATERNAL BEHAVIOR IN RATS. M. Numan, M.J. Numan and C.E. Lupini. Dept. of Psychology and Zoology-Physiology, Univ. of Wyoming, Laramie, WY 82070.

The present series of experiments explored the possibility that POA projections through the VTA to lower brainstem regions may be critical for maternal behavior in postpartum lactating rats. The first experiment found that bilateral coronal knife cuts posterior to the VTA depressed all aspects of maternal behavior, including nursing and building behavior. In a second experiment, it was found that a parasagittal unilateral knife cut lateral to the median eminence paired with a contralateral coronal knife cut posterior to the VTA also disrupted maternal behavior, suggesting that POA projections through the VTA to lower regions may be important. In a final anatomical study, animals received knife cuts posterior to the VTA or served as controls. Horsepreoxidase (HRP) was immediately injected into the knife cut region. Animals with the cuts had more HRP labeled cells in the POA than animals without such cuts, suggesting that the cuts did indeed never descending POA afferents. However, in the animals with cuts many other brain regions were also labeled with HRP. One functionally relevant area was the sensory trigeminal nucleus. This system may be particularly important for retrieving behavior which is influenced by crosssensory input.

Supported by a Whitman Foundation grant.

380.15
GUINEA PIG PERINEAL MOTONEURONS ARE SEXUALLY DIMORPHIC IN SIZE BUT NOT NUMBER. Louise M. Freeman & S. Marc Breedlove, Psychology Dept., U.C. Berkeley, Berkeley, CA 94720.

Motoneurons in the guinea pig innervating the bulbocavernosus (BC), levator ani (LA) and ischiocavernosus (IC) muscles of male guinea pigs are located in the ventral horn of the SI-L6 spinal cord. Caudally these neurons form a single cluster, but more rostrally they separate into two distinct motoneuronal columns, with BC and LA MNs in the medial cluster and IC MNs in the lateral group. Female guinea pigs have a much smaller cluster than the LA of males; MNs of this muscle, like those of the male LA, are seen predominantly in the medial column. There is no obvious IC in adult female guinea pigs.

To determine whether dimorphism in number or size, we examined ten spinal cord sections from intact adult males of each sex. Cords were frozen sectioned at 50μm and alternate sections mounted and stained with thionin. The caudalmost section in which the medial and lateral motoneuronal columns were distinguishable was chosen as anchor point; MNs of the medial and lateral clusters were counted. Counts were also made of the "preplit" cluster for 10 sections caudal to the anchor point. Raw counts were corrected for split nuclei by the method of Konigsmark. Nuclear and soma areas were measured from camera lucida drawings of 10-15 clusters/animal. There was a sex difference in somatic area and raw MN count. However, the latter difference was not of the same magnitude as the difference in size since the correction procedure eliminated the difference in MN number.

**MEAN SOMA AREA ± SEM (μ)**

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Precipitated Soma</th>
<th>Medial Lateral</th>
<th>Lateral Lateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>924±44</td>
<td>981±47</td>
<td>997±44</td>
</tr>
<tr>
<td>Females</td>
<td>786±24</td>
<td>799±24</td>
<td>813±32</td>
</tr>
</tbody>
</table>

2-way ANOVA showed no sex difference in MN size (p>.01) but no (p=.81).

Supported by a NSF postdoctoral fellowship (LMP) and March of Dimes (SMB).

380.16
ANDROGEN DOES NOT INCREASE MOTONEURONAL UPTAKE OF CT-HRP. Marianne Leslie & S. Marc Breedlove, Psychology Department, U.C. Berkeley, Berkeley, CA 94720.

It has been suggested that systemic androgen may augment choleterol toxin-conjugated horseradish peroxidase (CT-HRP) uptake by motoneurons in hormone-sensitive systems. We tested this possibility by gonadectomizing 38 male rats at 50-56 days of age, and implanting of silastic capsules filled with testosterone (T) or blank (B), 25-44 days later, CT-HRP was injected into 2 muscles; the left side of the hormone-sensitive bulbocavernosus muscle (BC) (2ul), innervated by the spinal nucleus of the CB (SNB) and the right flaxor digitorum brevis (FDB) (1ul), innervated by the retromesenteral nucleus (RDLN). Animals were killed 6-22 hours later, perfused, spinal cords frozen sectioned at 50μm, and motoneurons containing HRP reaction product counted by nucleus and by intensity of labeling (heavy or light). 

**Mean Total # Cells Labelled (heavy & light, both muscles):**

<table>
<thead>
<tr>
<th>Group</th>
<th>8</th>
<th>10</th>
<th>13</th>
<th>16</th>
<th>19</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

2-way ANOVA of heavily labeled SNB and RDLN motoneurons also reveals a significant effect of time but not hormone.

We found no evidence that systemic androgen could increase the rate of CT-HRP arrival in motoneurons; two potential sources of artifact when using CT-HRP to label dendrites.

380.17
PELVIC AND PUDENDAL NERVES INFLUENCE PACED MATING AND POSTURAL ADJUSTMENT IN FEMALE RATS. M.S. Erkine and E. Konrumb, Dept. of Biology, Boston University, Boston, MA 02215.

The present studies examined the relative contributions of the pelvic and pudendal genitounary nerves to lordosis behavior, to the pacing of mating stimulation, and to the postural adjustments required for receipt of cervical-vaginal stimulation in estrous female rats during mating. Bilateral surgical transection of the pelvic (Pe), pudendal (Pu) and pelvic (Pe+Pu) nerves, or sham surgery (Sh) was performed on ovariectomized Long-Evan's rats. Lordosis, pacing, and postural adjustment were measured after the sequential administration of estradiol benzoate (EB), silastic capsules and progesterone (P, 2 mg/kg) in Experiment 1 and after 7 days of injections of EB only (8μg/kg) in Experiment 2. In both experiments, high levels of lordosis behavior were seen in all groups. Pacing of mating stimulation was disrupted in Pe and Pe+Pu groups after EB and P; these females failed to show the pattern of withdrawal from males which in Pu and Sh groups reflected a disparity between medial and lateral proprioceptive inputs (l). After EB only, Pu animals as well as Pe and Pe+Pu failed to show discriminatory responses to M and I. Thus, the pelvic nerves are necessary for the learning of pacing behavior, postural adjustment, indicated by the ratio M/I, was similar in all groups given EB and P in Experiment 1, but after only M+I was elevated in the Pe and Pe+Pu groups. Thus, neither the pelvic nor the pudendal a. are required for postural adjustment if P is present; without P, intact pelvic a. are necessary for the female to make postural adjustments which increase the likelihood of intromission. HD31802.

380.18
LOCAL SITE OF ACTION FOR DEXAMETHASONE'S CATABOLIC EFFECT ON RAT BULBOCAVERNOSUS. Mark N. Rand and S. Marc Breedlove, Psychology Dept., U.C. Berkeley, Berkeley, CA 94720.

We previously found that local treatment of adult rat bulbocavernosus (BC) with testosterone can maintain muscle weight in ovariectomized animals; i.e. steroid capsules placed directly on the muscle show a local anabolic effect (Rand & Breedlove, Soc. Neurosci. Abstr. 1987). Last year we reported that the dendritic arbors of motoneurons innervating such T-treated muscles are 75% larger than those of motoneurons innervating the contralateral BC (p=.04, one-tailed). We now report that local treatment of BC muscle with the synthetic glucocorticoid Dexamethasone (Dex) results in a local catabolic effect on the muscle; our analysis of motoneuronal dendritic arbor length is pending.

Gonadally intact adult male rats 90-180 days of age were implanted with two capsules containing Dexamethasone (Sigma), and the other capsule contained PBS. Capsules were sutured on the surface of the muscle, one on one side of the BC, the other on the contralateral side. After 30 days the animals were sacrificed and the BC, spinal cord, and spinal neurons were dissected. The left and right halves were then weighed and weighed without knowledge of their steroid treatment. Muscle half weights were compared using a repeated measure t-test.

Muscle halves receiving left Dex were 10% lighter than those given blank capsules (p<.0003), indicating that Dex acts at or near the muscle for its catabolic effect.

The effect of this non-androgenic muscle-size manipulation on the length of SNB dendrites should be interesting. If dendrites of motoneurons innervating the Dex-treated muscle half are shorter, they would be shown to vary independently of normal androgen levels. If there is no difference in dendritic length with Dex treatment, then muscle size per se would be shown to vary independently of dendritic arbor. We are currently measuring SNB dendrites to test these two possible outcomes.

Supported by NSF #BSR 8451387.
381.1

COMMISSURE FORMATION IN SIX MOUSE STRAINS WITH ABSENT CORPUS CALLOSUM. D. W. Haltstein and B. Bulman-Fleming. Dept. of Psychology, Univ. of Alberta, Edmonton, AB. T6G 2E9 and Dept. of Psychology and Health Studies, Univ. of Waterloo, Waterloo, ON, N2L 3G1, Canada.

The study sought evidence of single gene effects on forebrain commissural development. Brains were studied morphometrically in fetuses of 13 inbred and one hybrid mouse strains ranging from 0.2 g to 1.0 g body size. Among six strains known to suffer absence of the corpus callosum (CC) in the adult, there was an association between a) percentage of adults with total CC absence and b) degree of retarded formation of the hippocampal commissure. The rank order of strains on both measures was BALB/c (worst), 129/Sv, C57Bl/6, DBA/2, C57Bl/6j and C3H/BlBy. This association occurs because in these strains the CC axons usually traverse the midline via the HC. The cross-sectional area of the medial septal region at the telencephalic midplane was greatly reduced in certain strains (e.g., 129/Sv and BALB/c) but close to normal in others (DBA/2 and BALB/cJ). These results support previous findings that there are two genetically and developmentally distinct processes which contribute to absent CC (Waltstein and Smith, J. Hirnforsch., 30:11-16, 1989). The causes of CC absence reside not in the CC axons themselves but in either the generation or support of cells that form the substrate for axon guidance. Supported by NSERC grant OGP004525.

381.2

A NEW MOUSE MODEL OF PARTIAL AND COMPLETE AGENESIS OF THE CORPUS CALLOSUM. H. P. Lipp, R. W. Wandera? and D. F. Wolfert. Institute of Anatomy, University of Zurich, CH-8091 Zurich, Switzerland.

Available animal models of callosal agenesis (the mouse strains BALB/c, 129/Sv and DBA/2) have the disadvantage of demonstrating a low frequency of this trait. The recently discovered mouse strain IN shows a complete absence of the corpus callosum (personal communication, R. E. Winer), but has low viability and is only available in small numbers. In order to obtain a complementary mouse model with high callosal variability and high frequency of CC defects, we studied the changes in size of the corpus callosum after crossing male in-mice with C57Bl/6j females and backcrossing the female offspring.

Changes in mid sagittal cross-sectional area of the forebrain commissures were assessed in brains of adult mice (4-8 months of age), 20 per generation. Groups included F1-hybrid (F1), 3 randomly mated generations (F2, F3, F4) and 3 generations from consecutively backcrossing female hybrid mice to the males of the parental strain IN (BX1, BX2, BX3). Area of the corpus callosum changed little in the F2-F4 groups (92-96% as compared to the F1). In the backcross generations, the corpus callosum was increasingly reduced with every generation (BX1: 76%, BX2: 58%, BX3: 59%). This corresponded to the number of mice with apparently complete agenesis of the corpus callosum (BX1: 0%, BX2: 45% and BX3: 75%). The reduction of the corpus callosum was correlated with a smaller area of the anterior commissure (r=0.58, p<0.001) and of the hippocampal commissures (r=0.86, p<0.0001), predominantly of their dorsal component.

Thus, variation in size and frequency of agenesis of the corpus callosum can be controlled by means of systemic breeding. Functional and neuroanatomical correlates of callosal variation are studied preferentially in BX2 mice, whereas BX3 mice combine decent viability with a high frequency of complete agenesis of the corpus callosum. Supp. by SNF Grant 3100-009470.

381.3


To study interocular transfer (IOT), we depend on directing sensory input to a single hemisphere. In studies of cats and monkeys, division of the optic chiasma routes all visual inflow in monocular animals to the ipsilateral hemisphere, enabling tests of IOT by section of forebrain commissures. Hemispheric asymmetries have also been investigated in this preparation. In the rat, section of the optic chiasma is formidable, and the alternative has been to exploit the marked asymmetry of crossed and uncrossed projections. Since the small proportion of uncrossed optic fibres in the rat is functional, the role of forebrain commissures in IOT is not firmly established. We describe a stereotactic technique using a microknife to transect the optic chiasma in the rat that minimizes superficial damage and mortality, and we present illustrative data on the interocular transfer of visual discrimination in chiasm-sectioned and chiasm-plus-forebrain commissure-sectioned animals. Chiasm-sectioned rats are visually competent and show mean savings (75%) on second-eye transfer. Rats with divided chiasma and corpus callosum show no evidence of transfer on first-eye reversal.

381.4

Behavioral asymmetry in male, female and perinatally gonadectomized rats. K. J. Schultz. Psychology Department, Univ. of Winnipeg, Winnipeg, MB, Canada R3N 2E9.

Behavioral asymmetry was examined in 31 Sprague-Dawley rats (105 males, 109 females, 103 perinatally gonadectomized males). Adult animals completed four trials on each of seven side preference tasks. Laterality ratios were derived for each task. A direct discriminant function analysis was performed using these laterality ratios as predictors of condition (male, female, gonadectomized male). The discriminant functions derived significantly separated males from females (p < 0.0001) but failed to differentiate between the groups of males (p = .613). The loading matrix values suggest that the best behavioral predictors for distinguishing between males and females were two circling tasks, a direction choice task and a water bottle preference task. On each of these tasks females exhibited a greater right preference than did males. None of the behavioral tasks discriminated between males and gonadectomized males. Analyses of hemispheric differences in neocortical volume are in progress and suggest that groups differ significantly on these variables.

381.5


We have previously reported that right hemisphere or bilateral suction lesions of the dorsal lateral frontal cortex or electrolytic lesions of the nucleus accumbens (NAS) in male rats produced spontaneous running wheel hyperactivity. In the present experiments, we examined these two phenomena in female rats. Twenty-four four adult female Sprague-Dawley rats were given either left, right, bilateral or sham frontal cortical suction lesions and placed in running wheel cages for activity monitoring for 30 days. The results showed no significant between-group differences in locomotor activity, demonstrating, in combination with our earlier findings in male rats, the presence of sexual disinhibition in the lateralized behavioral response to fronto-cortical lesions. In the second series of experiments, 27 adult female Sprague-Dawley rats were given either left, right, bilateral or sham NAS electrolytic lesions and activity was monitored as above. The results showed that rats with either left or right NAS lesions had significantly more locomotor activity than rats receiving either sham treatment or bilateral lesions. These results also contrast with our earlier findings in male rats where right but not left NAS lesions produced significant hyperactivity. Moreover, in adult male rats, bilateral NAS lesions produced more hyperactivity than single right NAS lesions.

381.6

ANTERIOR BRAIN ELECTRICAL ASYMMETRIES IN RESPONSE TO REWARD AND PUNISHMENT. S. Sobota, R.J. Davidson*, and J. Senuilis. Department of Psychology, University of Wisconsin, Madison, WI 53706.

Recent findings on both brain-damaged and normal individuals indicate differential involvement of the anterior regions of the two hemispheres in approach and withdrawal-related emotional processes. In this study, 15 right-handed subjects were tested in a paradigm which manipulated reward and punishment rates for correct and incorrect responses while brain electrical activity was recorded. Each trial began with the presentation of a fixation point, which was followed by an arrow which was either in the up or down position. Four seconds later, an imperative stimulus was presented. In the arrow up trials, if the subjects responded sufficiently quickly, they received $2.5; in the arrow down trials, if the subjects responded too slowly, they lost $2.5. RT criteria were adjusted for each trial block based upon the median RT of the previous trial block. After they responded, subjects were given feedback about their performance in trial. Trial type was randomly varied. EEG was recorded from F3, F4, F7, P8, T3, T4, C3, C4, O1, O2, Pz, Cz and sites between T3 & P3 and T4 & P4, referenced to A1-A2. Power in the theta, alpha, and beta bands of the EEG during the 4 s inter-stimulus interval was computed by Fourier Transformation. In addition, the contingent negative variation (CNV) was quantified during this interval. For alpha power, the Condition X Hemisphere Interaction was significant for the frontal leads. Punishment trials were associated with less right frontal power (i.e., more activation) compared with reward trials. These effects were present only in the frontal leads and were more robust when the analysis was restricted to trials on which the subjects won or lost money. No significant Condition X Hemisphere interaction was found for the CNV.

Damage to central dopamine (DA) systems in rats impairs aspects of spatial learning in the Morris water maze task. As the DA system is thought to play a preferentially medial role in spatial processing, we sought to determine if selective damage to the left or right nigrostriatal DA systems of rats would differentially impact the profile of spatial learning. Male Sprague-Dawley rats (350-350 g) were trained with unilateral 6-OHDA lesions of the left or right substantia nigra (N=24 per group) or control operations. Performance was based on a chart for directional bias (undrugged) in a 2 x 2 diameter pool, also used for subsequent water maze testing. Following a motor score test, rats were placed in a stereotaxic apparatus (AP+2 mm) with a 2-weak post lesion latency, rats were tested for acquisition of a place task (latency to find a submerged platform) in the water maze, for 4 consecutive days. On day 5 (reversal), the location of the platform was rotated to the opposite quadrant. Rats received 4 trials a day (water temp. 25°C). Only in reversal, did group differences emerge. Right-lesioned rats were significantly impaired in escape latencies (P=0.016) while left-lesioned rats did not differ from controls. In controls, a leftward reversal bias was correlated with time spent in the former escape quadrant in reversal (r=56, P=0.018), suggesting right-sided DA dominance for superior retention, a conclusion supported by significant correlations of APO rotation with the same measure in lefted groups. (Supported by NSERC. H.S is a Research Associate of the CHF).

381.8 SPATIAL DEFICITS AND THEIR LATERALIZATION FOLLOWING UNILATERAL POSTERIOR PARITIAL CORTICAL LESIONS IN RATS. S.E. Maier, R.W. Vigo3, M.F. Novotny & D.P. Crowe. Dept. Psychology, Univ. Waterloo, Waterloo, Ont. N2L 3G1, CANADA.

Both egocentric and allocentric (landmark) cues serve in spatial mapping and navigation. In humans, monkeys, and rats posterior parietal cortex (PPC) is involved in maintaining a reference system to guide spatial movement. Recent studies have implicated PPC yield allocentric deficits. Spatial deficits from unilateral lesions, especially in rats, are less well documented. To show a true homology of PPC function, we tested rats with unilateral lesions on egocentric and allocentric spatial tasks. Rats sustained left or right PPC lesions, lateralized PPC lesions + section of corpus callosum, CC section alone, or sham operations, and were tested on egocentric and allocentric versions of the Morris Water Maze. Both left and right PPC + CC lesions resulted in significant deficits compared to controls on latency and heading error in egocentric place navigation. With reversal of platform location, only controls and animals with left or right PPC lesions were impaired. On the allocentric task, rats with right, but not left, PPC + CC lesions were significantly worse than controls in latency to find the platform. We demonstrate a nonlateralized PPC impairment and a lateralized one in allocentric spatial navigation.


In cats, electrophysiological and tracing studies of the primary auditory cortex as well as the corpus callosum suggested the latter's implication in sound localization (Lenz, 1966; Poier et al., 1989). The present study examined manual pointing to auditory targets in 4 Ss with callosal agenesis paired to 4 age- and I.Q.- matched controls. In the latter's group, 30° (BBN) at fixed intensity in the horizontal plane in an anechoic chamber. BBNs were delivered randomly through 16 speakers and at repeated times. The Ss were blindfolded but visible to the Ss, were mounted at approximately 10° intervals on a perimeter frame having a radius of 30 cm. Ss responses consisted of manual pointing on the frame. Aiming accuracy was assessed by calculating the mean deviation of the pointing responses from the target position. There was no difference between the 2 groups in terms of accuracy. However, acallosal Ss tended to overshoot throughout their movements in both hemifields. The former group pointed symmetrically. Ss were therefore less accurate when aiming was carried out in the left auditory hemifield. The hemifield differences were not due to sensory deficits in sound localization. This conclusion is supported by visuo-motor pointing task with humans which showed similar differences (Lassonde et al. 1989). (Supported by FCAR and CRNSO).


In man the ease and accuracy with which we remember locations of objects after moving about without vision is taken for granted. When an object is viewed in one hemifield, and the subject rotates so as to "magnify" the remembered location into the opposite hemifield, does the neural representation of that locus jump from one side of the brain to the other? We performed a relevant experiment with four subjects with congenital agenesis of the callosum who could point to remembered visual targets (an eye-level cylinder seen for only 1/4 second) with either hand and in either hemifield. When memory locations remained within the same hemifield all subjects easily discriminated different locations; when the remembered location fell into the opposite hemifield after rotation locations of 30° vs 60° from the midline were very poorly discriminated. These results support the hypothesis that the underlying memory representations normally do change hemispheres post rotation, since good compensation occurred in controls equated for I.Q.

381.11 EFFECTS OF SEX AND AGE ON REGIONAL MORPHOLOGY OF THE HUMAN CORPUS CALLOSUM. P.E. Jakubowicz, S. Montal, A. Zaloga*, and V.M. Denenberg. Biobehavioral Sciences Graduate Program, Univ. of Connecticut, Storrs, CT 06269 and Dept. of Anatomy and Cell Biology, UCLA School of Medicine, Los Angeles, CA 90024.

Mid sagittal callosal (CC) tracings from MNI's of 73 pairs of age-matched males and females (age 2-79 years) were digitized using computer assisted software. The following parameters were derived: 99 percent points along the curved, maximum length, length of this axis, area, and perimeter. The width percentiles were grouped into regions based upon prior factors analyses of human callosal material. The regions are widths 3-18 (W3-18), W3-20, W5-22, W6-48, W8-94, and W9-99. Trend analyses of these regions with Sex and Age (blocked in 10-year bins) as independent variables, found the following significant effects. The two anterior most regions (W3-18 and W5-22) had a cubic Sex x Age interaction. In both regions males reached a maximum value at Age 21-30 while females did not peak until Age 41-50. In W3-18 both sexes dropped sharply after peaking. Their callosal widths after age 60 were very high. The same phenomenon was seen for females in region W22-39, while male widths remained asymptotic over age. The remaining callosal regions were relatively homogenous: the CC of both sexes grew with age (quadratic effect) and showed some growth diminution but no sharp drop off. The genu and anterior body were the CC regions most sensitive to the interactive effects of sex and age.

381.12 CORRESPONDENCE OF AUDITORY AND VISUAL LATERAL ASYMMETRIES FOR LANGUAGE PROCESSING IN DEXTALS BUT NOT IN NON-DEXTALS. Z. Caramanos*, R.J. Zatorre and D. Büht. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2L1.

Cross-modal correlations of visual and auditory measures of hemispheric language dominance were obtained for 16 dextral and 30 non-dextral human subjects. The auditory measure, ear advantage on the dichotic fused rhymed words test (S. Weisker & T. Hallas, Neuropsychologia, 21:119, 1983) was known to relate to individual hemispheric asymmetry on the intracarotid sodium Amytal test (R. Zatorre, Neuropsychologia, 27:1207, 1989). The visual measure involves the difference between the slopes of mean reaction times to words presented in the left and right visual fields as a function of length, which is taken to reflect hemispheric differences in reading efficiency (D. Bub & J. Lezine, Brain Lang., 33:161, 1988). Both dextrals and non-dextrals showed the expected distributions of asymmetry on the two measures. Dextrals showed a significant correlation (r=.56) between scores on the two tests, suggesting that, to some extent, a common underlying factor underlay the asymmetries observed in the two modalities. Non-dextrals, however, did not demonstrate significant cross-modal correlations (r=.13), which may indicate a partial dissociation between visual and auditory laterality in the non-dextral population.
381.13 HAND AND SEX DIFFERENCES IN SYLVIAN FISSURE MORPHOLOGY S.F. Witelson and D.L. Kigar, Dept. of Psychiatry, McMaster Univ., Hamilton, Ont., Canada. L5N 3T5

Morphology and asymmetry in the Sylvian fissure (SF) in the human brain was studied by dividing it into 3 segments: anterior (A), horizontal (H), vertical (V). Segment perimeters in each hemisphere were measured in 67 brains (24 men, 43 women) from individuals whose hand preference [consistent-right-handed (CRH) and nonCRH] was tested prior to death. Marked asymmetry was observed: left (L) HSF was greater than right (R); R VSF was greater than L; ASF showed no asymmetry. Sex and hand factors were significant in HSF only. (1) Men had greater asymmetry than women. (2) Men had a greater HSF than women but only for L HSF. (3) Only among CRH did men have a greater HSF (asymmetry) than women, suggesting that hand has a greater effect in men. In summary, there are two complementary asymmetries in the SF (HSF & VSF). The influence of handedness in men supports the role of SF morphology in functional asymmetry. The sex difference in the association of SF anatomy and handedness is consistent with previous results that area of the isthmus of the corpus callosum varies with hand only in men (Witelson, 1989).

381.15 COORDINATED NONINVASIVE STUDIES (CNS) PROJECT. J.J. Lauter, Speech & Hearing Sciences, Univ. of Arizona, Tucson, AZ 85721

Several noninvasive methods for studying human brain structure and function are combined in a test battery to study aspects of behavior in the same subjects. Each individual is tested with behavioral methods, MEG, PET, qEEG, EEG, MEG. Current focus is on asymmetries for complex sounds. First each subject is trained in dichotic listening for two sound sets which evoke "opposite" asymmetries. Next brain anatomical asymmetries are measured with MRI, and a repeated measures auditory ERP series is done to define brainstem asymmetries. Then each individual subject is run on the two sound sets while being monitored with qEEG, then PET, then MEG. Results to date show: 1) good "internal consistency" comparing behavioral, anatomical, and physiological asymmetries within subjects; 2) individual differences in the specifics of the various asymmetries; and 3) agreement across subjects in the patterns of these asymmetry "profiles." Findings suggest that the approach is not only viable, but that exploiting the complementarity of the noninvasive techniques may reveal unsuspected relations among aspects of human neuroanatomy, neurophysiology, and behavior.

DRUGS OF ABUSE: OPIOIDS

382.1 CONDITIONED AND UNCONDITIONED MORPHINE WITHDRAWAL IN THE HAMSTER. P. Schnur. Center for Alcohol and Addiction Studies, Brown University, Providence, RI 02904.

Naloxone-precipitated morphine withdrawal was studied in morphine-pelleted and in subcutaneously injected hamsters. On 5 days, morphine-pelleted (75 mg) hamsters were injected with naloxone (1 mg/kg) in a distinctive environment. They were observed for signs of withdrawal 10 min before and 30 min after the naloxone injection. Results indicated that a) withdrawal intensity was a direct function of the number of implanted pellets, and b) compared with several control groups, conditioned withdrawal developed among animals withdrawn in the distinctive environment and was evident up to 30 days after pellet removal. To extinguish conditioned withdrawal, some animals were given daily saline injections for 1 wk in the distinctive environment while others remained in the home cage. Conditioned withdrawal was attenuated, but not eliminated by the extinction treatment. A separate experiment indicated that verapamil (10 and 20 mg/kg) did not attenuate naloxone-precipitated withdrawal. In other experiments, withdrawal was precipitated by naloxone (0.1, 0.4, 1 mg/kg) following 4 or 8 sc injections of morphine (15 mg/kg). Withdrawal, measured as above, as well as by the disruption in normal food and water intake, was evident after 4 morphine injections. Again, verapamil failed to attenuate withdrawal. (Supported by NIDA Fellowship and by a Rhode Island Foundation Grant)

382.2 MORPHINE AND COCAINE-REGULATED PHOSPHOPROTEINS (MC-RPP) IN CRITICAL REWARD REGIONS OF RAT BRAIN IDENTIFICATION OF TYROSINE HYDROXYLASE (TH) AND OTHER DRUG-REGULATED PHOSPHOPROTEINS. D.B. Belmer and E.J. Nestler, Laboratory of Molecular Psychiatry, Deps. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06520.

The ventral tegmental area (VTA) and ventral striatal area (VTA) appear to mediate some of the rewarding properties of opiates, cocaine, and other drugs of abuse. In the present study, we have identified a number of phosphoproteins, among which is TH, that are regulated by both chronic morphine and cocaine specifically in these brain nuclei.

Protein phosphorylation was analyzed in brain regions of control rats and rats treated chronically with cocaine or morphine by back phosphorylation with cAMP-dependent protein kinase and 2D-gel electrophoresis. We found that chronic, but not acute, cocaine and morphine treatment regulated a number of the same phosphoproteins in the VTA and NAC. Both treatments increased the total amount of TH in the VTA, but reduced its phosphorylation state (without a change in its total volume). Several other, novel phosphoproteins also showed similar regulation by chronic morphine and cocaine in these brain regions. Drug regulation of most of these various phosphoproteins in the VTA was specific in that such regulation was not observed in the substantia nigra and striatum.

We propose that these Morphine and Cocaine-Regulated Phosphoproteins (MC-RPPs), together with increased adenylate cyclase and cAMP-dependent protein kinase activities observed in the NAC in response to chronic morphine or cocaine (see Nestler et al., at this volume), represent part of the biochemical basis by which these drugs of abuse alter the functional state of brain reward regions.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

We have shown that chronic morphine increases levels of IGF, Gov, adenylyl cyclase (AC), cyclic AMP-dependent protein kinase (PKA), and certain phosphoproteins (MAPPRPs) in the locus coeruleus, but not in some other brain regions studied (see J. Neurosci. 9:4371, 1989). These changes may occur at least in part at the level of gene expression. In the present investigation, we assessed the effects of chronic morphine or chronic cocaine treatments on the G-protein/cyclic AMP system in various brain regions. While most regions showed no regulation in response to morphine, locus accumbens (NAC) and amygdala did show increases in AC and CA-K, and thalamus showed an increase in AC only. More regulation of G-proteins was variable, with decreased levels of IGF and Gov seen in NAC, increased levels in amygdala, and no change in thalamus. Interestingly, chronic cocaine produced similar changes compared to morphine in G-proteins, AC, and CA-K in NAC, but not in the other brain regions studied.

These results indicate that changes in the G-protein/cyclic AMP system represent mechanisms by which a number of opiate-sensitive neurons adapt to chronic morphine and contribute to varying aspects of opiate tolerance and/or dependence in these cells. The findings that chronic morphine and cocaine produce similar changes in G-proteins, AC, CA-K, and phosphoproteins (see Beetner et al., this volume) in NAC, a brain region important for the reinforcing actions of abused substances, suggests further that changes in the G-protein/cyclic AMP system may also underlie psychological aspects of drug addiction.


We investigated the role of opponent processes in morphine tolerance by comparing morphine's effects on body temperature with those of LH secretion. All experiments used 4-day overtacclimated rats which were administered either a low or a high dose of morphine (5 or 30 mg/kg, s.c.) daily for 6 days. The low dose induced a transient suppression of LH levels (for 0.5 h) following the first exposure. Both responses showed little change with repeated injections. The high dose induced hypothermia and a sustained suppression of LH (up to 6 h) following the first exposure. After the sixth injection of 30 mg/kg morphine, LH levels were still suppressed to levels similar to the initial hypothermia. With the LH response, tolerance to the high dose was exhibited by an accelerated rate of recovery to normal LH levels rather than an attenuated suppression of LH. These results are best explained by an opponent-process account of morphine tolerance.

Our present investigations indicate that luteinizing hormone-releasing hormone (LHRH) attenuates morphine's effects on various response systems by inducing responses which oppose those of morphine. The present results are consistent with the hypothesis that enhanced LHRH release may mediate tolerance to morphine. (Supported in part by AG 05012)

382.7 VERAPAMIL REDUCES HYPERCAPNIA AND EUPHORIA PRODUCED BY MORPHINE IN HUMANS. D.B. Vapafl, A. Della-Papa, W.R. Lapos, and E.D. London. NIDA Addiction Research Center, Baltimore, MD 21224.

Verapamil (V), a calcium channel blocker, antagonizes morphine (M)-induced respiratory depression in rats (Rakocy, J. et al., J. Pharmacol. Exp. Ther., 238:192, 1986). As such an antagonist might be useful therapeutically, we extended the earlier work to a study in human volunteers. We tested the ability of V to reduce M-induced respiratory depression and to modulate subjective effects of M in four experienced, male heroin users. Five treatments, each consisting of 20 min infusion, were included: saline (S) + S; S + 10 mg S; S + 10 mg V; S + 2.5, 5, or 10 mg C. Carbon dioxide tension in the blood was measured transcutaneously (PcO2) and respiratory frequency was determined by impedance pneumography. Questionnaires and visual interval scales were used to evaluate subjective changes. Observations were made from 0.5 hr before to 4 hr after drug administration. M significantly increased PcO2 levels above S + S levels (P < 0.05), whereas none of the increases in PcO2 following M + V combinations differed from S + S condition. No consistent effects on respiratory rates were found. M produced slight to moderate subjective effects, the most common of which included self-reports of "good effects," "liking" and of being "high." These marginally significant effects (P < 0.1) occurred in 3 of 8 subjects. Additionally, all three doses of V consistently reduced the positive effects of M on mood, as well as the strength of the drug effect. Although further studies are indicated, the present results suggest that co-administration of calcium channel antagonist drugs, such as V, may improve safety and reduce abuse liability of M treatments.


It has been suggested that the peptide FLIYQFCRHR2 (P-6 to -HR2) may play a role in opiate dependence and subsequent abstinence syndrome. This peptide proved to be a competitive antagonist of the opiate abstinence syndrome, while IGP from antiserum against this peptide largely prevented subsequent naloxone-precipitated abstinence signs in dependent rats. The present study involved YFLQFCRHR2 (daR-8-to-HR2) was synthesized as a possible P-6-to-HR2 antagonist. It appears to be a mixed agonist/antagonist. At doses of 2.2g or higher it precipitates abstinence syndrome in dependent rats in a manner similar to P-6 to -HR2. However, 600mg i.c.v. did not induce withdrawal and attenuated naloxone-precipitated withdrawal signs in dependent rats. The peptide was ineffective in YFLQFCRHR2 (daR-8-to-NH2) i.e. while 7 received saline alone. Twenty-eight minutes later, all rats were challenged with 100mg/kg naloxone. Fifteen rats were cannulated in the 3rd ventricle and rendered dependent by 7 days continuous s.c. infusion of morphine sulfate (0.3 mg/mg/hr). via Alzet osmotic minipumps. Seven rats were injected with daR-8-to-NH2 i.c.v. while 7 received saline alone. Twenty-eight minutes later, all rats were challenged with 100mg/kg naloxone. In addition. During the following 20 min., each rat was observed under "blind" conditions for standard abstinence signs. The daR-8-to-NH2-pretreated rats showed signs of withdrawal compared to saline-pretreated controls, a significant difference, t < .001. There were significant differences in writhes/qaes, shakes/tremors, teeth chatter/chewing and pcxcls.


Previous studies have demonstrated genetic differences in various inbred strains of rats during preference tests and opopan x self-administration of ethanol, amphetamine and various opiate derivatives (George and Goldberg, 1989). Lewis (LEW) rats self-administered and displayed a preference for these drugs, while Fischer 344 rat did not. In the present study, the effects of morphine (10 mg/kg, i.v.) on EEG and behavior were examined and compared in these two strains. Duration of morphine-induced EEG slow-wave bursts and associated behavioral stupor was greater in LEW rats. Latency to onset of slow-wave sleep after morphine injection was also greater in LEW rats. Effects of morphine on EEG spectral parameters are being assessed. These EEG effects may reflect differences in neurosensitivity and/or opioid receptor populations. (Supported by NIDA DA01050 and NIAAA AA07754).
382.9 BUPRENORPHINE-INDUCED TOLERANCE TO DISCRIMINATIVE STIMULUS EFFECTS OF MORPHINE AND BUPRENORPHINE. G. Kapitopoulos* and A.M. Young, Department of Psychology, Wayne State University, Detroit, MI 48202.

The effects of acute or repeated treatment with buprenorphine (BUP) were examined in Sprague-Dawley rats trained to discriminate saline and 3.2 mg/kg morphine (MS) under a two lever fixed-ratio 15 schedule of food delivery. Cumulative doses of MS (0.13-3.2 mg/kg) or BUP (0.001-0.32 mg/kg) evoked MS-appropriate behavior in a dose-dependent manner, with complete generalization at 1.78 mg/kg MS or 0.032 mg/kg BUP. Acute saline BUP given 30 min (0.003-0.01 mg/kg) or 24 hr (0.1-0.3 mg/kg) before a test decreased the dose of MS or BUP required for stimulus control in an additive manner. In contrast, suspending training and administering repeated doses of BUP (0.32 mg/kg BUP) for 4 weeks increased the dose of MS or BUP required for stimulus control by at least 3-fold. However, during repeated BUP treatment, MS did not evoke stimulus control in 3 of 8 rats, and BUP did not evoke MS-like stimulus control in 2 of 8 rats. Repeated BUP treatment increased the dose of MS required for rate suppression by >6-fold, and that of BUP by >30-fold. Stimulus control by BUP recovered within 1 week after BUP treatment ended; stimulus control by MS, within 2 weeks. These results suggest that repeated treatment with BUP produces a prolonged tolerance to the MS-like stimulus effects of MS and BUP. (Supported by USPHS grants DA03796 and KO2 DA00132.)

382.10 HEROIN SELF-MANAGEMENT ON A PROGRESSIVE RATIO SCHEDULE OF REINFORCEMENT: THE EFFECT OF DOSE MANIPULATION, OPiate ANTAGONISM, AND SALINE REPLACEMENT. S.A.L. Bennett and D.C.S. Roberts, Department of Psychology, Carleton University, Ottawa, Ont., K1S 5B6.

Heroin self-management was examined using a progressive ratio schedule of reinforcement. On the first test day, the response requirements began at 1 and escalated exponentially after each drug infusion. On the second and subsequent test days, the initial response requirements were adjusted according to the previous day's final ratio. An inverted U dose response relationship was established for animals self-administering 12.5, 25, and 100 µg intramuscular heroin with breaking points peaking at the 50 µg/injection dose. Breaking points were shown to significantly increase following saline substitution for heroin injection and remain elevated for several days before extinction of the self-administration response was observed. Heroin-reinforced breaking points were shown to decrease rapidly following daily IP naltrexone (0.6 mg/kg) pretreatment. These results suggest that progressive ratio schedules of reinforcement are useful in quantifying changes in opiate-motivated behavior (Supported by the M.R.C.).

382.11 ULTRASONIC VOCALIZATION AS A MEASURE OF LONG-TERM AND SHORT-TERM TOLERANCE AND WITHDRAWAL IN NEONATAL RATS. C.P. Crapper, R.A. Place, and M.S. Panagis, Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Ultrasonic vocalizations are typically reduced by acute administration of opiates (eg. Kohn & Blas, 1986). We compared this behavior to nociceptive responses (pawlift on a hotplate) and used it as an index for studying the development of tolerance to and withdrawal from opiates in neonatal rats.

In 3 week old F-344 rats, morphine sulfate or saline were given once daily for the first 10 postpartum days essentially eliminated vocalizations compared to saline controls on each day. On Day 11, pups from each treatment group were administered saline, morphine (1 mg/kg) or naloxone hydrochloride (10 mg/kg). The morphine-exposed groups were as vocally depressed by the test injection of morphine as was the saline control group. Naloxone did not increase vocalization in the drug-exposed groups. Thus, unlike adults, neonatal rats did not develop tolerance and withdrawal using what have been termed "long-term" parameters. An alternative form, "short-term" tolerance, has been proposed for rats given closely-spaced doses of opioid (Baker & Tiffany, 1985). To test this form, pups received morphine (10 mg/kg) or saline at 6 hr intervals for 2 days, beginning on Day 1 or Day 10. They were then given saline, morphine (1 mg/kg) or naloxone (10 mg/kg) 6 or 36 hr after the last exposure and tested using ultrasonic vocalization and pawlift latency. In general, newborn rats appeared to be capable of acquiring tolerance and withdrawal if given massed injections of morphine in high doses. The apparent distinction between infant rat's ability to form long- and short-term tolerance suggests that the mechanisms subserving each form of tolerance develop at different ages.


Three high doses of morphine will produce oral stereotypes in rats that can be re-expressed up to 6 months later. Because amphetamine-induced oral dyskinesias are often dependent on the mode and setting of drug delivery, the present study determined the role of these variables in morphine-induced stereotypy. Male F-344 rats were continuously injected with morphine (30 mg/kg intraperitoneal, IP) over 36 hours, or were injected with 3 high doses of morphine (10,20,20 mg/kg, sc) over 24 hours. Throughout the morphine administration, rats were placed in test chambers with steel bar floors. Thirty days later animals received morphine (4.0 mg/kg) and were observed for evidence of oral stereotypy. There was no significant difference in the incidence of stereotype between the two groups. In a second study rats received 3 high doses of morphine and were placed in either the test chambers with floor bars or in a solid plastic box. Thirty days later, all animals were injected with morphine (4.0 mg/kg) and were placed in the chambers with bar floors. All animals showed oral stereotypy. Unlike amphetamine-induced oral dyskinesias, the stereotypies produced by morphine appear to be obligatory. (Supported by NIDA grant DA03236 and RSA DA00099 to CK).

382.13 THE ROLE OF NIGRAL OPIOID RECEPTORS IN MEDIATING MORPHINE WITHDRAWAL. H. Nagy and A.A. Baumeister, Department of Psychology, Louisiana State University, Baton Rouge, LA 70803.

These studies were conducted to investigate the role of opioid receptor subtypes in the substantia nigra in behavioral symptoms of morphine withdrawal. Male Sprague-Dawley rats were given daily injections of increasing doses of morphine sulfate (20-80 mg/kg/i.m., s.c.), or saline (5% mineral oil:42.5% Arlacel A:50% saline). Eighteen hours after the last morphine injection, animals received bilateral intranigral injections of 5nM/0.5 nl naloxone (nonspecific antagonist), beta-FNA (mu specific antagonist), nor-BNI ( kappa specific antagonist), or sterile water (0.5 nl). Withdrawal behaviors were observed for 20 minutes following intranigral injection. Naloxone produced significant increases in wet dog shakes, ptosis, teeth chattering and squawling (irritability to touch), when compared to the intranigral water control. The only effects produced by beta-FNA and nor-BNI were an increase in teeth chattering and squawling respectively. Ptosis and squawling were significantly increased by naloxone compared to beta-FNA and nor-BNI. These data suggest teeth and wet dog shakes in morphine-dependent rats are mediated by nigral mu and kappa receptors, respectively, whereas squawling and ptosis are not mediated exclusively by these receptor subtypes.

382.14 Electrophysiological identification of Accumbens (NAS) neurons by evoked responses to multiple afferent stimulation explains variance of NAS opioid drug effects. P. L. Hakan* and J. Huirink, University of North Carolina-Wilmington & Research Inst. of the Scripps Clinic, La Jolla, Ca. 92037

The effects of systemic morphine on fimbria-evoked NAS neuronal responses in anesthetized rats have been described as heterosynaptic (1993, Huirink and Lehn). As part of ongoing studies of the effects of opioids on NAS neuronal activity we currently report that the effects of systemically administered opiates on ventral pallidum (VP)-evoked NAS neuronal activity are also heterosynaptic. However, concurrent stimulation of VP and fimbria input to the NAS have shown patterns of convergence and nonconvergence that do not vary across different drug categories. NAS neuronal activity was recorded in a group of functionally identifying distinct NAS neuronal populations (Hakan & Huirink, 1999). Further analysis of population effects in this light has been in contrast to earlier observations of heterogeneous opiate actions. For some NAS neurons, many of the effects are largely uniform. For example, these NAS neurons that are monosynaptically driven by both fimbria and VP stimulation are consistently inhibited by systemic morphine. The effects of opiates on other NAS subpopulations are still apparently mixed. It is predicted that further functional categorization of these populations by afferent inputs will ultimately explain the remaining variance of opiate effects in this region.
382.15 

Ibogaine, an indolealkylamine, has been claimed to abolish the drug craving of a few heroin and cocaine addicts for at least 6 months (patent No. 4,449,096) and, in preliminary studies in this laboratory, one to five doses of ibogaine (40 mg/kg) has decreased I.V. morphine self-administration by rats for several days thereafter. Drugs of abuse belonging to different pharmacological classes have been shown to stimulate dopamine (DA) transmission in the mesolimbic system; this is believed to be the basis for the rewarding effects of those drugs. The present study used the microdialysis technique to determine the effects of a single dose of ibogaine on basal extracellular DA levels and also the effects of ibogaine pretreatment on dopamine stimulation of brain DA systems. Ibogaine (40 mg/kg, IP) decreased DA extra-cellular levels in nucleus accumbens and striatum and increased DA levels in the prefrontal cortex. When injected 19 hr prior to a morphine challenge (5 mg/kg, IP), ibogaine (40 mg/kg, IP) prevented the rise in DA levels in the striatum and nucleus accumbens observed after morphine injection alone. These results are consistent with the proposed use of ibogaine in the treatment of opiate addiction. (Supported in part by NDA International, Inc. and NIDA grant DA03817).

382.17 
INCREASED D1 AND D2 RECEPTOR ACTIVITY DURING OPIATE WITHDRAWAL: BEHAVIORAL AND BINDING EVIDENCE. J.W. Tidery and R.A. Micek. Dept. of Psychology, Tufts University, Medford, MA 02155.

The mesolimbic DA system is most likely affected by opiate withdrawal; converging evidence particularly implicates altered dopaminergic activity in these behavioral effects. Previously, we reported that the administration of d-amphetamine to morphine-withdrawn rats results in high levels of aggression which outlast motoric withdrawal signs. We have investigated this effect further by administering the selective D1 and D2 agonist SKF 38393 (100 mg/kg) and quinpirole (0.1-1 mg/kg) to mice which had undergone removal of s.c. morphine or placebo pellets at varying time points before evaluation of both motor activity and aggressive behavior toward an intruder. The D1 agonist SKF 38393 decreased aggressive behaviors in both morphine-withdrawn and placebo mice without affecting rearing, walking or grooming. The D2 agonist quinpirole maintained elevated levels of attack, threat, and motor behavior in morphine withdrawn mice while all of these behaviors were suppressed in placebo controls. A similar behavioral profile was seen in mice who were administered 3.0 mg/kg SKF 38393 before quinpirole injection. To determine whether these behavioral changes are due to upregulation of dopamine receptors during withdrawal, D1 and D2 binding of 3H-raclopride and 3H-SCH 23390 was measured in vivo in the striatum, olfactory tubercles and cerebellum of mice which had undergone morphine or placebo pellet removal 5, 48, or 96 hours previously. Morphine-withdrawn mice showed high levels of D1 and D2 receptor binding as compared to placebo controls; this increased D1 and D2 receptor occupancy during withdrawal may be relevant to the heightened aggressive behavior.

382.18 
SOMATODENDRITIC RELEASE OF DOPAMINE IN THE VENTRAL TEGMENTAL AREA FOLLOWING MORPHINE. M.A. Kiltenick and F.W. Kalivas. Department of VCAEP, Washington State University, Pullman, WA 99164-6520.

A number of recent studies have suggested a modulatory role for opioids and GABA in the mesolimbic, A10 (VTA) dopaminergic system. Opiate injection into the VTA elicits an increase in dopamine (DA) metabolism and locomotor activity, the latter of which can be blocked by DA antagonists. Repeated administration of morphine results in a progressive increase in the evoked behavioral response in addition to a decrease in DA utilization within the VTA. GABA has also been shown to regulate DA transmission. Injection of a GABA agonist into the VTA activates the mesolimbic DA system, while a GABA antagonist inhibits DA activity.

In order to further assess the suggested modulatory role of opioids and GABA on the mesolimbic DA system, in vivo dialysis was used to measure DA and its metabolites in the VTA following administration of morphine (1 and 10 µM) or baclofen (1 and 10 µM) through the dialysis probe. Administration of baclofen through the probe had no effect on DA levels within the VTA; however, morphine was found to elicit a dose-dependent increase in the somatodendritic release of DA, DOPAC and HVA.

382.19 

Intracranial self-administration of opioid agonists which are selective for the µ and δ receptor subtypes was demonstrated in independent groups of male Long-Evans rats. Each animal was unilaterally implanted with a chronic guide cannula and was allowed to lever-press for ventral tegmental area (VTA) injections of one of three compounds: morphine (mixed agonist), DAGO (selective µ agonist), or DPDPE (selective δ agonist). Each compound was effective in establishing and maintaining a lever-pressing habit in animals with VTA cannulae; however, animals with dorsal control cannulae did not learn to lever-press. These results raise the possibility that µ and δ receptors in the VTA are involved in the rewarding effects of opiates.
383.1

Depts. of Surgery, Anatomy & Neurobiology, and Pathology, University of Tennessee, Memphis, TN 38163.

Neuroanatomical Ca accumulation (EICA) is a fundamental pathogenic event in muscular dystrophy (Bhattacharya & Johnson, Neurology India, 37:145, 1989). Concomitant to EICA, elevated CK, histopathology, cardiac dysfunction, and reduced lifespan are common in Duchenne muscular dystrophy (DMD). We have shown striking similarities in pathogenesis between DMD and BIO-146 DH (Muscle & Nerve, 10:168, 1987). Since BIO-146 DH are no longer available, we investigated C80-146 strain male DH from Canadian Hybrid Farms, Nova Scotia, as a potential model for DMD. C80-146 strain albino normal hamsters (NH) served as controls. EICA, measured by atomic absorption spectroscopy, was profound in the cardiac and skeletal muscle of DH. Plasma CK and relative cardiac enlargement were also elevated. Among the most noticeable histologic changes in DH were the abundance of atrophic, hypertrophic, and necrotic fibers with fatty infiltration, central necrosis, foci of disarray, and fibrosis. EM of myocardium revealed mitochoniral Ca deposition and loss of myofilaments. Increased mortality in DH ensues at 90 days of age with average life expectancy of 150 days, compared to 3 years for NH. We conclude that C80-146 strain DH is an excellent model for therapeutic screening in DMD. Supported by HDA and NIH Grant AR-38540.

383.3

CONTRIBUTIONS OF GENOTYPE TO CEREBELLAR MORPHOLOGY: DIFFERENCES IN PRIMARY FISSURE DISTORTION IN INBRED STRAINS OF RATS. G. B. Ward, C. Goodwin, L. M. Nichols*, and J. R. West. Dept. of Anatomy, University of Iowa, Iowa City, IA 52242.

Aberrant development of the primary cerebellar fissure in rats, including fusion is the depth of the fissure, and presence of ectopic neurones, has been reported in a number of studies of outbred rats. Substantial variation in the frequency and extent of such distortion has been found both within and between outbred strains. In this study, we examined the primary fissure of eight inbred rat strains: ACI, Brown Norway (BN), Buffalo (BUFF), Fisher 344 (F344), Maudeley Reactive (MR), Marshall albino White Norway (WN) and Wistar-Kyoto (WKY). All subjects were derived from a breeding colony maintained in our laboratory, originally established using breeding pairs obtained from NIH. Saggital sections, either 10-μm frozen sections or 2-μm JB-4 sections were taken through the cerebellum, stained with a Nissl stain, and examined microscopically. Several strains (BN, F344, MR) exhibited severe distortions of the primary fissure within the vermis, with partial fusion of the fissure and loss of the ependymal cell layer. Others (ACI, M520, BUFF) had mild to moderate distortion, usually limited to partial fusion of the primary fissure, with only occasional ectopias. Little or no distortion of the primary fissure was seen in the remaining two strains (WN, WKY).

Regardless of strain, these perturbations were generally more pronounced in the 10-day-old subjects and other differences were found in cerebellar structure, ranging from ectopic cells in lobule IX (M520) to dramatically increased gyration (BN). Although variation in the extent of distortion was observed within strains, the marked individual differences in severity were predominantly accounted for by differences among strains. These findings demonstrate differences among inbred strains in the morphological development of the cerebellum, and suggest that studies of inbred rats may provide a useful model to evaluate the contribution of genotype to aberrant development of cerebellar fissures and lobules. (Supported by Grant AA-07313)

383.7

THE TWITCHER MOUSE: IN VITRO STUDIES OF SCHWANN CELLS (SC) OR ADHESIVENESS AND RATE OF PROLIFERATION. A. Koninčan and K. Suzuki. Department of Pathology, School of Med., Univ. of North Carolina, Chapel Hill, NC 27514.

A previous study of isolated SC from twitcher revealed apparent differences in the rate of cell growth between sucking and adult mice (Iino and Suzuki, 1990). To elucidate the cause of such differences, we examined adhesiveness and rate of proliferation of SC in vitro. SC were isolated from sciatic nerves and brachial plexus of three genotypes (twi/twi, twi+/+ or sc) at P10 (group 1) or P30 (group 2) and were cultured (3 X 10⁶ cells/12cm² coverslip). Cell number and [H] thymidine uptake of the SC were determined at 1, 2, 4, 6, and 8 days in vitro (DIV). SC adhesiveness at DIV 1 in group 1 was similar in all genotypes. The cell number and [H] thymidine uptake of the SC were determined at 1, 2, 4, 6, 8, and 12 DIV in vitro. SC adhesiveness at DIV1 in group 2 was higher than any genotype in group 1. The cell number and [H] thymidine uptake of the SC were determined at 2, 4, 6, 8, 12 DIV in group 2. In contrast, SC adhesiveness in twi/twi in group 2 was higher than any genotype in group 2. The cell number and [H] thymidine uptake of the SC were determined at 2, 4, 6, 8, 12 DIV in group 2. SC adhesiveness, which was most pronounced but thymidine incorporation remained low. The results indicate that 1) hypofunction or dysfunction exists in twi/twi compared to twi+/+ in vitro, and 2) the apparently accelerated cellular proliferation in adult twi/twi is a result of increased adhesiveness of the SC secondary to demyelination in adult twi/twi nerves.

383.8

EVALUATION OF TRENOR IN A NEUROLOGICAL MUTANT OF SPAGHR-DALEY RATS. Roger E., Vega-J., Holmgren B., and Vega-Navarro de Menne E. Dept. de Clinica Fisiologica ICSUP, Univ. Autonoma de Puebla. P. O. Box 406, Puebla, Mexico.

In the rat colony of our laboratory, a neurological mutation appeared, characterized by tremor, ataxia, tonic immobility episodes and paralysis. The initial neurological sign is a fine tremor in the tail and hindlimbs which is visible at 1 month age. Later, the syndrome is transmitted by autosomal recessive inheritance. Tremor frequency was recorded in rats from 15 days to 12 months old, using a power spectral analysis following a procedure described by Shinohaski (Neurosci. Res. 2:63-76, 1984). One month old rats exhibited tremor at an average frequency of 7±0.3 Hz; at the age of 2 months and until 12 months the main peak frequency is at 5±0.4 Hz. Tremor is attenuated in the administration of the dopaminergic antagonistic spenadmine (APO) (0.01-0.1 mg/kg), effect which is not observed with higher doses of APO (0.5-1) nor with the dopaminergic antagonist sulpiride (30-100 mg/kg). The underlying mechanisms of this hereditary neurological syndrome is under current study by our group.

383.4

TESTS OF GENETIC ALLELISM BETWEEN FOUR INBRED MOUSE STRAINS WITH ABSENT CORPUS CALLOSUM. D.J. Livy and D. Wahlsten. Dept of Psychology, University of Waterloo, Ontario, N2L 3G1, Canada.

University of Alberta, Edmonton, Alberta, T6G 2E9 (Canada).

If two inbred strains show absence of the corpus callosum (CC) because they have the same single locus, data from them should also have CC absence. If the hybrid is normal, the parent strains must differ at one or more significant loci. Over 450 weaning mice were obtained from the four inbred strains and 11 hybrid crosses, and their brains were sectioned sagittally and stained for myelin. All crosses with the normal strain C57BL/6J were normal, demonstrating no loci for inheritance. When the CC strain (100% total CC absence) was crossed with severely affected strains (BALB/cWahl1, BALB/cWahl2, 129ReJ), hybrid mice with a BALB/c parent showed numerous CC defects, whereas those with a 129 parent had less severe defects than 129. Crossing either BALB/c strain with 129 yielded offspring with or without very few CC defects. Crossing the two BALB/c strains produced mice resembling BALB/cWahl2. It is concluded that the strains BALB/cWahl1, BALB/cWahl2 and 129 have essentially the same genetic abnormality, differing only in severity of the defective alleles, but that 129ReJ differs from these three strains, most likely at a single locus.

Supported in part by an NSERC postgraduate scholarship to DJL and operating grant to DW. Thanks to Kathryn Blom for doing the histology.

383.6

CHANGES OF NEUROEPITHEDES IN SPINAL CORD AND BRAINSTEM OF WOBBLE MOUSE AT DIFFERENT STAGES OF MOTONEURON DISEASE. K.Ki. Tanig. F.Tanig. I.L. Vacca-Galloway. Dept. of Anatomy and Physiology1, Fac. of Med. Univ. of Hong Kong, Hong Kong.

As a model for study of human motoneuron disease, the autosomal recessive mutant Wobblc mouse (wr) exhibits a progressive loss of motoneurons in spinal cord and brainstem. In early stages of the disease, axons containing substance P (SP) and thyrotric releasing hormone (TRH) but not leucine enkephalin (LE) appeared increased by immunocytochemistry. This phenomenon is observed (Vacca-Galloway, L.L. and Steinerber, C. J. Neurosci. Res., 16:657-670, 1986). Recent radioimmunoassay (RIA) studies have shown that TRH, LE and methionine enkephalin (ME) contents increased in the spinal cord at different stages of the wr disease (Tanig, F. et al., Brain Res., in press, 1990). In the present study stages of the motoneuron disease were evaluated in NRK myc neurones (MN, Reseda, MA), 3 weeks to 11 months old, identified by behavioural test (Langen, D.J. et al, J. Neurosci., 61:211-216, 1983). The wr mice (Stages 1 to 4) and their controls were evaluated for the presence or absence of the spinal cord, brainstem, also hypophalamic and midbrain where axons are collected, and the TRH, SP, LE and ME contents were compared by RIA.

In experiments wherein control values were pooled, TRH in spinal cord was increased significantly in Stages 1 and 2, SP increased in Stages 1 and 4, and LE increased throughout Stages 1 to 4. When control litters were statistically paired with wr litters, SP contents at Stage 1 showed no increase in spinal cord. ME contents increased in Stages 1 and 2 but not 3 and 4 when control values were pooled, but showed opposite results when control and wr genotypes were compared. In wr brachial cord, TRH increased significantly in Stages 2 and 3, however SP showed no increase. ME and LE became increased in Stages 3 and 4. Trends observed for these peptides in spinal cord and brainstem may relate to disease progression. Supported by awards from USPHS (338/0130006) and Croucher Foundation (36001/0814).

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
383.7
ABERRANT DEVELOPMENTAL REGULATION OF TYROSINE HYDROXYLASE EXPRESSION IN TOTTERING AND LEANER
MOUSE MUTANTS. E.J. Hash, III, M.C. Wilson. Dept. of Neuro-
parmacology, Research Institute of Scripps Clinic, La Jolla, CA, 1993.

The inherited autosomal recessive mutations, tottering (gene symbol: t) and leaner (gene symbol: l), have neuronal abnormalities of a single gene locus in mice. The tottering mouse mutant exhibits spontaneous ataxic seizures, focal motor seizures and mild hindlimb ataxia while the leaner congenic mouse traitor displays severe ataxia and no focal motor seizures. We have previously reported ecstatic expression of tyrosine hydroxylase (TH) in the cerebellar Purkinje cells of the adult mouse mutants tottering and leaner (Soc. Neurosci. Abstr. 15:986, 1989). In order to assess the onset of ecistic TH expression, TH mRNA expression was examined by in situ hybridization on postnatal day 1 (P1) through P28 tottering and leaner mouse mutants and age-matched heterozygous littermates. Hybridization of the 25S-rRNA TH probe was detected in the locus coeruleus and substantia nigra in all mice from P1 through adulthood. TH expression in the Purkinje cells of tottering and leaner mice was first observed at P18 and persisted through adulthood. On P18, TH expression was also observed in the Purkinje cells of the heterozygous control mice and in wild type C57Bl/6j mice; the TH expression in Purkinje cells of control mice persisted through P28 and was absent in adult mice. These data indicate that TH is normally transiently expressed in Purkinje cells during postnatal development, similar to transient developmental TH expression observed in other brain regions, such as the inferior colliculus. Moreover, these data suggest that tottering and leaner mouse mutants are deficient in the signal which suppresses TH expression in adult Purkinje cells. The identification of such a deficiency is crucial in understanding and characterizing the t gene. Supported by PHS CA33750, NS32038 and an American Epilepsy Society Research Fellowship.

383.8
MEANDER TAIL: GENETIC AND HISTOLOGICAL ANALYSIS OF A MOUSE MUTATION WHICH MAY DEFINE A DEVELOPMENTAL DEPARTMENT IN THE HUMAN CEREBELLUM. Galin Elston, Carol Mason, Mary Beth Hartman and Nathaniel Heitz. Rockefeller University and University of Miami, College of Physicians and Surgeons, NY, NY, 10021.

As part of a comprehensive program to identify the genes responsible for several aberrant phenotypes in the mouse, we have adopted a molecular genetic approach to clone the gene product of the Meander tail mouse. Meander tail (maz) is a recessive mutation mapping to mouse chromosome 4, whose phenotype is characterized by both neurological and skeletal abnormalities. Thus, the adult maz/maz mouse displays normal foliation and cytoarchitecture in the posterior lobes of the cerebellar cortex. However, starting with an abrupt transition, Purkinje cells are completely disorganized in the anterior lobes; their anterior extent varies in all directions, including into the white matter. Granule cells are sharply reduced in number at this transition and rapidly become indiscernible. Additionally, gial cell processes extend in all directions.

The maz locus has been fine mapped on chromosome 4 by analysis of several hundred progeny of an interspecific backcross to Mus carasuan. Closely linked markers are being used to isolate mouse YAC clones for the purpose of walking toward the locus.

The striking transition from normal to grossly disrupted cytoarchitecture involving at least three cell types in the meaz/meaz cerebellum suggests the presence of a discrete developmental compartment in the anterior cere-
bellum.

383.10

The objective of this present study was to determine the topographical distribution of dopamine (DA), choline and GABA uptake in the striatum of control (+/+) and mutant (wv/wv) mice. Uptake of [3H]-DA, [3H]-choline and [14C]-GABA was determined in sucrase homogenates prepared from the dorsolateral (DL), dorsomedial (DM), ventrolateral (VL) and ventromedial (VM) portions of the striatum from +/+ and wv/wv mice. In 45-60 day old control mice, DA uptake was homogeneously distributed throughout the striatum. This distribution was altered in the wv/wv with the ventral aspects being less severely affected than the dorsal regions. In wv/wv mice, the uptake was exhibited significantly reduced uptake rates. In 9 and 12 month old mice uptake higher was in lateral than in medial aspects of the striatum of both genotypes with no differences being observed between genotypes. GABA uptake was evenly distributed in both genotypes, and again no differences were observed between +/+ and wv/wv mice. The present observations indicate that the entire wv mutation is severely deficient in its ability to reuptake DA and is thus functionally compromised. The results also indicate that striatal cholinergic and GABAergic interneurons are not directly or indirectly affected by the wv gene. (Supported by ROI NS 14426).

383.11

Weaver is an autosomal recessive mutation in mice that results in cell death among the dopamine-containing mesencephalic neurons and in neuronal cell death in postnatal development. Previous observations made both of cerebellar granule cells and the dopamine-containing cells suggest that the gene affects neurite development. This hypothesis is supported also by the present comparison of tyrosine hydroxylase (TH)-stained cells in the midbrains of 7-day old mice of three genetic types: homozygous normal, heterozygous weaver and homozygous weaver. The examination was made before the onset of most of the cell death that takes place in the mesencephalon of the normal mouse. We saw a dramatic lack of TH-positive dendrites in homozygous weaver mice in areas of pars reticulata adjacent to densely packed TH-positive cell bodies. The weaver's midbrain compared with the midbrain of the normal mouse in which TH-positive dendrites formed a curtain of tightly aligned processes in the pars reticulata. In the heterozygous weaver in which dopamine-containing cells do not die, the pars reticulata was nevertheless only sparsely filled with dendrites.

The results show that the gene's effects on dendrites precede the death of vulnerable cells and that a single dose of the gene affects dendrite formation without causing cell death. Supported by NS20181 and MH100655.

383.12
TWO GENES FOR TEMPORAL LOBE EPILEPSY MAPPED IN THE MOUSE. M.L. Rice, W.N. Franklin*, J.M. Cotten* and T.N. Seyfried, Dept. of Biology, Boston College, Chestnut Hill, MA 02167, and Dept. of Molecular Biology, Tufts Univ. School of Medicine, Boston, MA 02111.

El is a neurological mutant strain of mice which is considered a model for complex partial seizures in humans (Seyfried, Elston, 124:143, 1985). Two F1 hybrid populations were generated from the following reciprocal crosses: El x ABP/L and El x D2. The F1 progeny from both crosses were screened using three previously established genetic markers that define the El and D2 parental parents. The offspring of both BCs were divided into El-like (seizure-susceptible) and D2-like (seizure-resistant) groups. Inheritance of El is determined by a single nuclear marker. Both groups display a wide variety of complex partial seizures, including absence, complex partial and tonic-clonic. Both groups show a wide variety of complex partial seizures, including absence, complex partial and tonic-clonic. The former association was between El and the chromosome 1 marker m14 and d4, whereas D2-like mice were p2 and ABP/L-like. The two loci displayed no significant linkage. These results suggest that El and D2 loci may be a significant linkage. These results suggest that El and D2 loci are located on the same chromosome. This is in agreement with the findings of previous studies.
384.1

THE RELATIONSHIP OF INFARCT SIZE TO BLOOD PRESSURE AND TIME OF OCCLUSION DURING (PHARMACOLOGICALLY INDUCED) THERMIC STROKE IN-REVERSAL. ED Watson, H. Kamitake, S. Fardo, W.D. Dietrich, M.D. Ginsberg, Neurology, University of Miami, Miami, FL USA

The middle cerebral artery of halothane-anesthetized mature male Sprague-Dawley rats was exposed and irrigated with three independently positionable 457.9 nm laser beams. In order to lower the power threshold for thrombus formation and enhance lysis by t-PA, bis-(a singlet oxygen quencher) was injected with flavin mononucleotide (FMN) at 13.4-660 ul 1.3-2.0 wt respectively. Two beams positioned distal to the rhinal branch and the other just below it, infarct area (A) at epicenter was proportional to the time of occlusion (t) in mm of blood pressure range of 90-110 mmHg for beams focussed sharply across the arterial diameter (0.9x0.8 mm, 40 min/c4 hr, n = 7, r = 0.96). Diffuse beam focussing enhanced clot stability (1.3x0.7 mm, 3 hr/c4 hr, n = 5, r = 0.48). At higher pressures (115 to 130 mmHg and diffuse focussing, A and t were not correlated. This real time evidence that infarct susceptibility to thrombotic stroke is fundamentally conditioned by arterial blood pressure and collateralization. The rheological and histopathological characteristics of this minimally invasive and reconfigurable (by endogenous or exogenous t-PA) model of arterial thrombotic stroke should provide insights into human clinical stroke.

384.3

POSTISCHEMIA (S)-EMOPAMIL THERAPY AMELIORATES FOCAL ISCHEMIC INJURY. E. Horikawa, M.D. Ginsberg, W.D. Dietrich, and R. Busto. Cerebral Vascular Disease Research, Univ. of Miami, MI 33101.

In a previous study, (S)-emopamil (E), a calcium-channel blocker and serotonin 5 antagonist, reduced cortical infarct volume in rat middle cerebral artery occlusion (MCA-O) (Nakayama et al, Neurology 38:1667, 1988). In this study, we explored the temporal window of this thrombotic efficacy forty-four in male Sprague-Dawley rats under permanent proximal right MCA-O plus 30 min halothane-induced hypotension to 50 mm Hg (Osborn et al, JNEN 30:402, 1987). There were 2 experimental groups: A) E-treatment groups: Group 1 (n = 9) received E 20-30 min prior to MCA-O; Group 2 (n = 13), 2 (n = 12), and 3 (n = 8) received E 1, 2, and 1 hours respectively, following MCA-O. Group 5 (n = 12) consisted of vehicle-injected controls. The initial E dose was 20 mg/kg IV with a 2nd dose 2.5 hr later and 1 hour after MCA-O. After 24 hr, brains were prepared for histologic quantitation of infarct area at 8 standard coronal levels; numeric integration yielded infarct volume. Cortical infarct volume in Groups 1-5, respectively, were in mm3, mean ± S.D.: Group 1, 53.4 ± 26.4; Group 2, 37.6 ± 27.6; Group 3, 47.8 ± 34.1; Group 4, 76.0 ± 26.9; Group 5, 52.9 ± 33.3. Striatal infarct volume was not affected. These data confirm a significant reduction of cortical area from 1-3 post-treatment and a trend at 2-4 hr, suggesting a temporal window of efficacy.

384.4


Our model of severe nerve ischemia consistently results in extinction of the compound nerve and muscle action potential (NAP; CMAP) within 30 minutes. Since impulse transmission and/or gene expression may depend on nerve energy metabolism (NEM), we studied the effects of ischemia with reperfusion on NEM in vivo, in vitro and reperfusion. Ischemia for 30 minutes postmortem or in deoxygenated Ringer's solution resulted in marked depletion of adenosine triphosphate (ATP), adenosine diphosphate (ADP), glucose (Glu), and creatine phosphate (CP) an increase in lactate (LAC) of sciatic-tibial nerve of adult male Sprague-Dawley rats. In vivo ischemia for up to 3 hours in sciatic-tibial nerve was sufficient to extinguish CMAP but did not deplete ATP, CP, or Glu and did not increase LAC. Ischemia sufficient to extinguish NAP (caudal nerve) resulted in reduction of energy substrates to about 50% of resting. Muscle fails to contract impulses but nerve and a trend reductions of energy substrates are milder than in vitro changes.

384.5

ISCHEMIA INDUCES RELEASE OF GLUTAMATE IN REGIONS WHICH ARE SPARED FROM HISTOPATHOLOGICAL DAMAGE. M.Y. Chiu, C. Gloeck, R. Busto, E. Martinez, I. Valdez, and W.D. Dietrich. Cerebral Vascular Disease Research Center, University of Miami, School of Medicine, Miami, FL 33101.

Excessive release of glutamate is thought to play a major role in the susceptibility of neurons to ischemia. In the present study we evaluated whether regional differences in the magnitude of glutamate release could explain selective vulnerability to ischemia-induced changes in extracellular levels of glutamate in a region selectively vulnerable to 10 min of transient ischemia (CA1 sector of the hippocampus) were compared to the changes occurring in regions which, although rendered ischemic, were not affected by a 10 min ischemic episode, comprised by the CA2-CA3 sectors. The degree of the ischemia and the histopathological outcome were also evaluated in these regions. Blood flow reduction and energy depletion were severe and uniform in all regions. The histopathological outcome illustrated a severely damaged CA1 sector of the hippocampus while all other brain regions were unaffected. Extracellular glutamate levels, measured by microdialysis, were significantly elevated during ischemia in all four regions. Glutamate levels continued to increase during the early reperfusion period and gradually returned to baseline by 30 min of reperfusion, with similar temporal changes in all regions. These results demonstrate that elevated intrasikhemic glutamate levels of themselves are insufficient to engender ischemic damage, and other factors may play a pivotal part in the detrimental role of glutamate during ischemia in vivo.

384.6


To compare the consequences of global ischemia due to multiple vs. single insults, we subjected rat to ischemia by either A) a single 15 min high-grade forebrain ischemic insult (n = 7), or B) three 5 min insults separated by one-hour intervals (n = 7) ischemia was produced by bilateral carotid artery occlusion plus moderate hypotension (50 mm Hg) in rats whose cranial and rectal temperatures were separately thermoregulated at 36 and 97°C respectively. Three days later, ischemic cell change (ICC) was quantitated. In hippocampus, caudoputamen, and cerebral neocortex, ICC was equally severe in Groups A and B. The ventrolateral thalami, in contrast, showed moderate-to-severe ischemic injury in all rats of the multiple-insult group (B) but in only 1 of 7 rats in the single-insult group (A); numbers of ischemic neurons per high-power field were 4.7 ± 2.2 (mean ± S.E.) in Group A, and 30.8 ± 4.0 in Group B (p < 0.01). Preliminary intra-cerebral microdialysis data revealed 1: a delayed massive increase in extracellular striatal glutamate levels associated with a) diminished extracellular glutamate 5-6 hours following the third multiple insult; b) findings not observed in the single-insult group. These results indicate cumulative effects of repeated normothermic insults. Supported by Grants NS22603, NS05820, and NS26784.

384.7

EFFECT OF ISCHEMIA AND REPERFUSION ON OXYGEN FREE RADICALS IN RAT SCIATIC-TIBIAL NERVES. K.K. Ward, J.D. Schmeler, N. Parmiani, K. W. Ben preprocess and P.A. Low. Neurophysiology Laboratory, Department of Neurology, Mayo Foundation, Rochester, MN 55905

Our model of severe nerve ischemia consistently results in extinction of the nerve impulse, reduced reflow and reperfusion injury to blood-replenished nerve fibers. We examined if oxygen free radicals (OFR) were generated during ischemia and reperfusion. Conjugated dienes (CD) and malondialdehyde (MDA) were measured in rat sciatic nerve. The endothelin:pienarin ratios for CD, MDA and OOH were 3.46, 0.16 and 0.93 respectively, indicating that CD was mainly in endothelium, OOH mainly in epineurium and MDA evenly distributed. In vitro reoxygenation resulted in no significant generation in OFR. Reoxygenation resulted in near doubling of MDA without changes in CD or OOH. A significant decrease in a reduction in OOH with a near 3-fold increase above the ischemic value on reperfusion. Smaller changes were examined in CD, with no changes. These findings suggest that OFR is generated during reperfusion rather than during ischemia.
ISCHEMIA IV


Extracellular striatal dopamine concentrations were measured using on-line repeating chronoamperometry in urethane anaesthetized rats before, during and after transient forebrain ischemia. Striato-modified carbon paste electrodes were implanted bilaterally into the striatum, and baseline extracellular dopamine was monitored for at least one hour. Ischemia was induced for 20 minutes by bilateral carotid occlusion combined with intracerebral hypotension to 80 mmHg. After this period, the carotids were unclamped and the shed blood reinfused over about 10 minutes. Extracellular dopamine levels fell after a characteristic initial rise within three minutes. The period clamping there was a 1.5-2 fold rise, followed by a gradual steady increase during the 20 minutes of occlusion. When the arterial clamps were removed there was an immediate decrease in the chronocoulometric signal. This was followed by a few minutes later by a second 1.5-2 fold increase. The second rise in extracellular dopamine lasted for approximately 40 minutes, after which the concentration dropped gradually to a stable, elevated level with respect to the initial preischemic baseline. We suggest that the initial rise in extracellular dopamine is due to ischemic depolarisation of the neuronal membrane, whereas the second, larger increase is a result of reperfusion. Either of these increases in extracellular dopamine may be involved in striatal neuron death following cerebral ischemia.


Transient forebrain ischemia (15 min) was produced in anesthetized rats and rats by bilateral carotid clamping coupled with reduction of blood pressure to 25 torr by rapid removal of blood. During ischemia the EEG became isoelectric and returned to normal levels once the carotids were unclamped and the blood reinflused. Animals were sacrificed at 7 days post-ischemia. In the post-ischemic period, rats had slower to recover from the acute procedure than rats and lost rather than gained weight. In rats 575% of hippocampal circumference from CA1 through CA3 suffered severe neuronal damage (>50%) compared to 42% in rats. In both sexes neuron losses in CA1 were severe (-35%) and less pronounced in CA3 (-15%) Glia proliferated in CA1 (>10-fold) and in CA3 (-3-fold); rod-shaped microglia were prominent in stratum radiatum, and astrocytes and oligodendroglia increased in strata pyramidal and oriens. Glialosis occurred in animals even when there was little or no neuronal loss. We conclude that CA3 rats are more vulnerable to ischemia than CA1 rats and that glial proliferation can occur in areas where only relatively mild neuronal losses are observed. Supported by NIH grant 5R17849 and the Veterans Administration.

384.11 TEMPORAL CHANGES DURING CEREBRAL EDEMA IN LACTATE AND PH MEASURED IN VIVO BY 1H/31P-NMR. S. Man-Bryce, G. Rosenberg. Neurology Service, Veterans Medical Center, and the Dept. of Neurology and Physiology, Univ. of N.M., Albuquerque, NM 87131.

Changes in brain metabolism during the development of brain edema were examined using in vivo 1H- and 31P-NMR with surface coils and depth pulses. We produced a colagenase-induced intracerebral hemorrhage in adult rats (Rosenberg et al., Stroke, 1990). Colagenase-treated animals had a longer onset to maximal edema in the posterior brain region, and a high behavioral injury score. Fifty-five animals had two NMR recordings each prior to injury and at 4, 8, 12, 14, 48, 72, and 144 hrs after injury. CA1 and CA4 regions of the left hippocampus were examined using a whole-body spectrometer. 1H spectra were used for peak height and lactate (1.3 ppm), N-acetyl aspartate (2.0 ppm), and PCR/Cr (3.0 ppm) monitoring. Initially, the chemical shift between 1H and PCR/Cr peak were used to determine the pH values from 31P spectra. Lactate was increased over pre-injury values at 4, 12, 24, 36, and 48 hrs (+p<0.05), but correspondingly, less familiar for all time points. However, the experiments indicate that an increase in brain lactate concentration may occur in the edematous region without a decrease in brain pH.


Both opioid agonists and antagonists have been shown to be beneficial in the treatment of ischemic brain injury. The current experiments determined the efficacy of CI-977 a novel, potent kappa-selective agonist, (-affinity 0.012 µM, Kappa agonist 50% loss) for reducing damage resulting from focal ischemia. The left common carotid artery of F-344 rats was ligated along with coagulation of the middle cerebral artery 3.7 mm above the basilar suture. CI-977 or vehicle (saline) was administered IV 30 min and 24 hr post occlusion. In one experiment animals were sacrificed 2 days post occlusion and the degree of damage assessed by exclusion of the dye TTC in 2 mm sections. CI-977 (0.05 to 0.5 mg/kg) produced dose-related reductions in infarct size which were significant at 0.1 and 0.5 mg/kg. In another experiment the animals were perfused fixed with 10% buffered formalin at 10 days after occlusion and sections were taken every 800 µ and stained with iron hematoxylin/cresyl-echt violet. CI-977, at 0.1 mg/kg significantly decreased the volume of ischemic damage to 57% levels. At 0.5 mg/kg CI-977 produced a non-significant decrease of 27%. These data suggest that CI-977 may be beneficial in the treatment of cerebral stroke.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

The opioid antagonist nalmefene, which has increased affinity for μ-opioid receptors, attenuates the release of glutamate in vivo following experimental global cerebral ischemia/reperfusion (Faden et al., in review). In the present study, the effect of nalmefene upon excitatory amino acid release during global ischemia was studied using the technique of microdialysis. A 4-mm microdialysis probe was stereotaxically placed in the dorsal hippocampus and perfused with mock CSF at 2 μL/min in 26 isolate anesthetized rats. One hour after insertion of the probe, common global cerebral ischemia was induced for 30 minutes by the 7 vessel occlusion method. Saline, 0.1 mg/kg of (-) nalmefene or the inactive (+) enantiomer was given 15 minutes prior to ischemia. Samples of dialysate were collected daily 10 minutes before, during, and after ischemia and amino acid content determined by HPLC. Peak dialysate glutamate during ischemia was significantly (P<0.05) less in (-) nalmefene treated rats (21.3±4.0 μM), while there was no difference between the saline (35.4±6.4 μM) and (+) nalmefene groups (34.9±5.0 μM). These results suggest nalmefene may modulate glutamate release during ischemia and that inhibition of excitotoxin release may contribute to the protective actions of opioid antagonists in cerebral ischemia.


FPL 13950, a novel pyrrole (FPL 10137-100, a 2-amino-N-(2,4-dihydro-3-methylbutyl)acetamide) is a moderately potent, safe, orally active anticonvulsant which protects rodents against maximal electroshock seizures (ED50=20.5 mg/kg i.p., in mice & 15.5 mg/kg i.p. in rats; Garske et al., Soc. Neurosci. 14: 866, 1988). During routine screening with thermostimulated mice, oral FPL 13950 extended the time to death during hypoxia (20+19). Analogue tests in rats were confirmatory. In global ischemia models rats were subjected to 30 min. of 4-vessel occlusion, followed by 20 mg/kg FPL 13950 or saline i.p., immediately after 20 min. of reflow and daily for 2 weeks. Drug treated had much less CA1 cell loss and smaller CA1 electrophysiological responses (orthorhodic & antidromic population spikes recorded in vitro) than saline-treated rats. Dogs were subjected to 8 min global ischemia (clamping the ascending aorta), treating with FPL 13950 i.v. 30 min. post ischemia, then b.i.d. for 3 days and then once daily for 7 days). Histological examination confirmed that FPL 13950 markedly reduced ischemic damage in CA1. These findings warrant continued development of this or similar compounds for eventual indications in patients suffering from cardiac arrest or coronary bypass surgery.


Glucocorticoids can potentiate ischemic neurotoxicity in the rat hippocampus. However, the cellular and biochemical mechanisms underlying this synergy are unknown. To begin to characterize this effect we have used primary hippocampal cultures, containing both neurons and astrocytes (1:1), derived from 16D fetuses at 10-12 days of age. Cultures were reseeded with experimental medium containing 5 mM glucose with or without 100 mM steroid. 24h later, cultures were reseeded with steroid-free medium containing varying glucose concentrations, immediately made toxic (100% N2) for 6 hr, then reseeded with fresh medium (0 mM glucose). The following day, cell damage was measured by lactate dehydrogenase assay. Pretreatment with corticosterone (CORT) enhanced hypoxic cell damage under low (0.4 mM) or no-glucose conditions but had no effect when glucose = 5 mM. In normoxic controls, CORT was damaging only in the 0-glucose group even though glucose-free exposure alone caused no significant damage. Neither progesterone, estrogen, nor testosterone had any measurable effect. Thus, CORT aggravates hypoxic injury in hippocampal cultures only under conditions that are likely to cause energy failure and subsequent cell death. These results are consistent with the idea that glucocorticoids enhance cell vulnerability by weakening cellular energy state. In support of this hypothesis, glucocorticoids have been shown to inhibit glucose transport into cultured hippocampal neurons and astrocytes. Supported by NIH AG-06633.


Hypertension is one of the most common risk factors for stroke. Spontaneous hypertensive rats (SHR) have been demonstrated to experience a high incidence of ischemic and hemorrhagic lesions in the brain stem, following a provocative dose of lipopolysaccharide (LPS). LPS stimulates macrophages to secrete the cytokines interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), both of which contribute to the production of tissue necrosis. In order to determine if increased TNF-α activity in the brains of SHR was responsible for the high incidence of ischemic lesions in SHR, we challenged SHR and normotensive rats (WKY) with 1.0 mg/kg LPS and measured TNF-α levels in the brain stem. We found that LPS induced a marked elevation of TNF-α activity in both SHR and WKY. However, the response of the SHR was significantly higher than that of the WKY controls, resulting in a significantly higher TNF-α level in the brain of SHR 2 hours following challenge with LPS. Furthermore, when LPS was delivered intravenously, TNF-α activity was elevated mainly in the brain but not in the CSF. By contrast, when LPS was injected intracerebroventricularly, TNF-α was high in the CSF and low in the blood. These results strongly suggest that TNF-α is produced locally in the brain and thus may cause local ischemic lesions. The higher incidence of stroke-like events occurring in SHR as compared to WKY may be related to the higher level of TNF-α obtained after challenge with LPS.
ACETAZOLAMIDE-INDUCED ERRORS IN ISCHEMIC I2O2/15 MINUTES AFTER ISCHEMIA.

23.6% of the rats were in a state of ictal epilepsy as measured by EEG, ECG, and intracerebral pressure monitoring. The EEG showed a consistent pattern of high-frequency, low-amplitude activity, with occasional spikes and waves. The ECG showed a prolonged QT interval, with a mean of 4.2 seconds. Intracerebral pressure measurements were also elevated, with a mean of 25 mmHg. These results indicate that the administration of isometric exercise to the ischemic tissue may contribute to the development of epilepsy in these rats.

384.20


Delayed neuronal death was induced in a rat known to cause G-Loc in pilots of high performance aircraft with potentially grave consequences. Relative little is known about G-Loc and so approaches to its prevention are limited. In the present study a small animal centrifuge (SAC) was used to investigate the neurophysiological effects of G-Loc. Rats with surgically implanted electrodes for ECG, EOG and heart rate were loaded on a SAC equipped with a freeze blowing device. Control rats received identical surgical treatment but were not centrifuged. Rats were centrifuged for 30g at 1 to 32.5g to determine G-Tolerance and time for G-Loc to occur. Brains were frozen immediately (group 1), or 0-60s after centrifugation and analyzed for energy metabolism. At 25 ± 1 g (n=19), G-Loc was observed within 7 ± 18 seconds. Brain glucose decreased (75%) in group 1 and remained unchanged in group 2 over controls. Brain lactate increased 2-2.5-fold over control in both groups. ATP and creatine phosphate levels decreased and AMP, ADP and adenosine levels increased significantly in both groups. This is the first report to show metabolic changes during G-Loc. Investigations continue to determine if these and other changes contribute to delayed neuronal death.

ISCHEMIA V

385.2


Selective vulnerability and "delayed neuronal death" of hippocampal CA1 region have been well documented, but its precise mechanism has not been clarified so far. Our previous report demonstrated that the degradation of the neurofilament (NF) triplet proteins occurs in cerebral ischemia and suggested that it may be resistant to irreversible neuronal cell death (Ogata et al., J. Neurosci., 7:103, 1989). In this study, we examined the expression of NF200, a major NF protein in pyramidal neurons, using immunohistochemistry. NF200 expression was significantly decreased in the CA1 region after global ischemia, indicating that the degradation of NF200 is associated with neuronal degeneration. This finding suggests that the NF200 protein is a sensitive marker for the detection of delayed neuronal death in ischemic brain injury.
RESPONSE OF SOMATOSTATIN NEURONS TO TRANSIENT FOREBRAIN ISCHEMIA IN THE GERbil. AN IN SITU HYBRIDIZATION STUDY. L. J. Heinecke*, B. E. Epstein, and C. S. Lue. Dept. of Neurology and Physiology & Biophysics, Hahnemann University, Philadelphia, PA 19102-1192.

The selective vulnerability of specific populations of forebrain neurons to ischemic insult has been well documented. These include neurons in the hippocampal CA1, CA3, and CA4 regions, the lateral septum, the striatum, and the neocortex. Recently, somatostatin positive neurons have been found in these regions. We therefore investigated the use of these somatostatin neurons to ischemic insult. Because different forms of the somatostatin molecule are derived from a single precursor and individual neurons may express all forms, an in situ hybridization procedure was used to localize neurons containing somatostatin mRNA.

ISCHEMIA

385.3

DISTRIBUTION OF 70 KD HEAT SHOCK PROTEIN mRNA INDUCTION AFTER TRANSIENT GLOBAL ISCHEMIA IN THE RAT. T. S. Newhall, Jr., G. Nappapiglia, F. Kawai*, and J. Katz. Div. of Laboratory of Neuropathology and Neuroanatomical Sciences, NINDS, NIH, Bethesda, MD 20892.

Recent studies have documented the induction of the 70 kDa stress / heat shock protein, hsp70, following transient ischemia in the gerbil, and have suggested its utility as an early marker for neuronal circuitry which may participate in the evolution of subsequent pathological changes. Since postischemic protein synthesis deficiencies may play a role in the production of the major neurotoxicity, these experiments were performed by follow-up reperfusion of 1, 3 or 7 days. Normal, untreated rats served as controls. The distribution of soluble proteins from these regions was examined for changes that might lead to cell death.

Male Wistar rats were subjected to 15 min. transient forebrain ischemia as early as 3d post-occlusion. Similar results were obtained with the 4d and 2w survival conditions. Furthermore, cell loss and argyrophilia were noted in the same regions stained with Cresyl Violet and silver, respectively. Therefore, the loss of somatostatin mRNA signal was most likely due to cell death. It remains possible, however, that surviving cells cease to express somatostatin mRNA. Our present results suggest a correspondence between the localization of somatostatin neurons to ischemic damage in the gerbil forebrain. Supported by NIH S07RR07241.

EXPRESSION OF HEAT SHOCK PROTEIN 70 mRNA FOLLOWING REVERSIBLE FOCAL ISCHEMIA IN RAT BRAIN. E.A. Welsh and D.J. Movius, Div. of Neurosurgery, Univ. of Pennsylvania, Philadelphia, PA 19104.

To investigate the relationship between the expression of heat shock proteins and ischemic brain damage, in situ hybridization was performed in postischemic rat forebrain using a complementary DNA probe specific for inducible 70-kd heat shock proteins (HSP-70). Reversible focal ischemia was produced by transtemporal craniectomy, and 1 hr of both carotid arteries with permanent occlusion of the middle cerebral artery. After reperfusion for 7 days, an intense expression of HSP-70 mRNA was evident in the area of ipsilateral neocortex known to undergo infarction. However, HSP-70 mRNA was also expressed in regions not normally injured, including medial neocortex, striatum, and hippocampus. After 24 hr reperfusion, expression of HSP-70 mRNA was still evident in neocortex and CA1 subfield of hippocampus, but not in striatum. These results demonstrate that the postischemic expression of HSP-70 mRNA is not region-specific but underlies ischemic injury and in regions that recover without histologic evidence of damage.


Ten minutes of four- vessel occlusion (4-W0) in the rat usually produces no striatal ischemic damage. We tested the effect of dialysis probe implantation and perfusion on the striatal cell loss produced by 10 min of 4-W0. Male Wistar rats were prepared for permanently cauterizing the ventricle arteries and placing an atrumatic snare loosely around the carotids. At the same time, a "loop type" micromedial dopamine (DA) probe was inserted at a rate of 1 µl/min with an artificial CSF solution containing 150 mEq NaCl, 3 mEq KCl, 1.7 mEq CaCl2 and 0.9 mEq MgCl2 with a pH of 7.4. After about 2 hours of perfusion, 4-W0 was produced in the animals by closing the snare around the carotids. Loss of righting reflex was used as an index of adequate ischemia. After 10 min. the snare was opened and reperfusion begun. At least 25 hours later, the rats were sacrificed and striatal damage was assessed histologically. Rats that were never an ischemic cell loss in the non-perfused striatum. In the perfused striatum, 10 of the 11 rats showed some cell loss and 5 of these showed severe cell loss symmetrically distributed around the dialysis membrane.

We have investigated cerebral ischemia following 5 min of total brain ischemia in gerbils. Before ischemia, 25-40% of the cerebral cortex and 15-30% of the striatum were labeled with K-111. Cerebral blood flow (CBF), plasma glucose, lactate, and pyruvate were measured continuously during and after ischemia. CBF decreased to 0 within 30 sec; lactate and pyruvate levels increased, and plasma glucose concentration fell to near zero. At 5 min after ischemia, all the parameters were decreased to zero.

On reperfusion, K-111 uptake was observed in the cortex and striatum at 1 min and gradually increased and returned to normal values at 15 min after ischemia. This suggests that ischemia is not permanent and that recovery is possible after ischemia.

385.15 SEGMENTAL BLOOD FLOW IN ISCHEMIC SPINAL CORD OF RABBITS. K.E. Peek, J. Goddard-Fiegold, M.J. Toergersen, C.S. Robertson. Dept. of Neurosurgery, Baylor College of Medicine, Houston, TX 77030.

Blood flow before, during, and after spinal cord ischemia may affect neurological recovery. Eight New Zealand White rabbits were anesthetized with ketamine and artificially ventilated with supplemental oxygen. A balloon catheter was positioned in the abdominal aorta just distal to the origins of the renal arteries. Somatosensory evoked potentials were monitored at L5-L6 during acute spinothalamic tracts. Functional spinal cord ischemia was determined by the disappearance of postspinal potential following ischemia. Simultaneously, blood flow in the spinocerebellar tracts (sCBF) was measured in the 9 caudal segments using 15-micron radiolabeled microspheres at 4 times: before and during ischemia at 10 and 15 min reperfusion.

Before ischemia, mean SD sCBF varied from 12.2+3.10 mL/100g/min at T12-L1 to 17.5±3.10 mL/100g/min at L7-S1. During ischemia, sCBF decreased to 31.7%±7.3% (L1-L2) and 93.9%±12.7% (L7-S1). At 10 and 15 min reperfusion, hyperemia was evident at all segments, with the greatest percent change from pre-ischemic values (75%±70%) at L5-S1. By 120 min reperfusion, sCBF values had normalized to control levels at all segments. sCBF may prove to be an important correlate in acute and chronic measures of functional recovery from spinal cord ischemia.

Using a photochemical method (Watson B. et al., Ann Neurol 17:497, 1985), focal thrombosis was generated in cortex (PCIs) of 3-mo old male F-344 rats. One wk after implantation of jugular catheters, experimen-
tal rats (n=7) were anesthetized by halothane; given scalp retraction; injected with 3 uN HgSO4 (200ug/kg); and then illuminated on cranium (trigemina coordinates: A-P=-1.6±0.4 mm; L=4.3 mm) with a high intensity light source for 40 min bilaterally. Controls (n=7) received identical treat-
ment except for dye injection and skull illumination. After 10 days rats were
pretrained in 1-way active avoidance to criterion (8 avoidances/10 trials), and
24 hr later received 15 trials in a 1-unit T-maze with performance sensitive to aging and septo-hippocampal damage (Ingram D., Neurobiol Aging, 8:475, 1986) as well as to aspiration lesions of PCIs during acquisition (Ber-
quencies and durations. In pretraining, no differences were observed in shock avoidance. Thus, the infarct primarily affected cognitive performance in the maze. Histological assessment showed infarct usually confined to PCIs with no hippocampal encroachment but occasional occipital cortex damage. Additional experiments using occipital cortex infarct will determine the signifi-
cance of this damage. However, results suggest PCIs involvement in the age-related learning deficit in this task may provide a model of cerebral ischemia with a well-defined functional impairment.

385.17 THE EFFECTS OF TRANSIENT ISCHEMIA ON THE MI MUCARINIC RECEPTOR IN GERRIL FOREBRAIN. G.R. Luthin and C.S. Lin, Department of Physiology and Biophysics and Institute for Neuroscience, Hahnemann University, Philadelphia, PA 19107.

Transient ischemia produces selective neuronal death in gerbil forebrain. The goal of this study was to establish the pattern of expression of muscarinic receptors in the adult male gerbil after ischemia. The ml subtype of muscarinic acetylcholine receptor (ml mAChR), following ischemia. This marker was chosen based on our recent observation that regions known to contain high levels of ml mAChRs are those most affected following ischemia. An anti-antipeptide antiserum was produced in rabbits, affinity purified and used to localize the ml mAChR in gerbil brain. Ischemia was produced by bilateral carotid occlusion (5 minutes). 3-7 days following the occlusion, gerbils were sacrificed, perfused with 3% paraformaldehyde, and brains were sectioned with a vibratome. Tissue was blocked with IOX normal donkey serum, incubated with primary and secondary antibodies, and the ml mAChRs were localized using DAB as substrate for HRP. The brain regions showing a decrease of ml mAChR staining directly paralleled those evidenced by staining with silver. These include the hippocampus, but not the dentate, and lateral portions of the caudate-putamen. These data indicate that there is a direct correspondence between the neuronal areas containing a high concentration of ml mAChRs and those most vulnerable to transient ischemia. (Supported by NS 23006 and NIH 5078207241)


The no-reflow phenomenon and its role in the pathophysiology of human stroke are poorly understood. No-reflow is seen in rat models of global cerebral ischemia incorporating cerebrovascular fluid compression or artificially induced hypotension, although the latter is more important. Furthermore, human stroke. Because the production of no-reflow is related to the severity of ischemia produced, models that produce no-reflow may be used to help distinguish neurochemical events of particular importance to survival. Thus, a neuro-


Exposure of the rat motor cortex to an intense light in the presence of a fluorescein dye initiates a sequence of events, including the local ischemia that mimics the events occurring during a stroke in man. We now report that it is possible to produce a bilateral infarct in the dorsal-medial prefrontal cerebral cortex of the rat which results in a reliable impairment of learning and memory performance.

Rats were trained to alternate responses between two levers. Intertrial (retention) intervals of 2.5, 5 and 10 sec were randomly distributed within each session. Upon reaching criterion rats were assigned to a Lesion or Sham group (n=8). The lesion was produced by administering the fluorescein dye rose bengal, i.e., exposing the skull, and centering a 4 mm diameter beam of high intensity light 2 mm anterior to Bregma for 60 minutes. The lesion produced a significant, retention interval-dependent impairment of alternation accuracy over the first post-operative week. Mean percent correct at the 2.5, 5 and 10 sec intervals was 97, 89, and 78 in the Sham and 59, 42 and 38 in the Lesion group. With continued testing, the magnitude of the deficit diminished. The lesion did not affect one measure of performance (probability of any response) but slightly increased the latency to a response (reaction time).

385.18 SILICONE CYLINDER EMBOLIZATION AS A RAT STROKE MODEL. A.D. Perez-Tropechko and S.C. Jones. Cerebrovascular Res. Lab., Cleveland Clinic Foundation, Cleveland, Ohio 44195.

Reliable stroke models are important for further investigation of the pathophysiology and treatment of stroke. Silicone cylinder embolization, in contrast to the middle cerebral artery ligation model, produces a focal lesion leading to a less traumatic model.

Seven male Sprague-Dawley rats weighing (293 ± 9 g, mean ± SEM) were anesthetized with pentobarbital (50 mg/kg ip). Body temperature was maintained at 37°C during the surgery. Unipolar EEG was recorded over the MCA region. The left common carotid artery (LCCA) was exposed and the proximal-
palatine artery electrocoagulated. The LCC was temporarily clamped, for less than 10 min to allow cannulation of the left common carotid artery (embo-lized) a silicone cylinder (900 μm long, 300 μm diameter) was then inserted into the LCCA. Two other rats received only normal saline. EEGs were obtained before and after embolization, under anesthesia and 24 hr later in the anesthetized state and were analyzed using Fourier frequency analysis. Peak frequencies were significantly different between the two groups, 3.60 Hz in the Sham and 4.15 Hz in the Lesion group. After 24 hr the animals were decapitated and 2 mm brain slices were obtained. The slices were incubated with 2,3,5-triphenyltetrazolium chloride in phosphate buffer at 40°C for 30 min and fixed in formalin. The infarct volume was determined using digitized image analysis for at least 8 photographed sections for each animal.

The infarct volume, expressed as percentage of total brain, was 10.65 ± 1.86%, n = 5 in embolized animals (mean ± SEM) and 0.15 ± 0.15%, n = 2 for the sham animals (mean ± range). The EEG analysis showed an immediate decrease in amplitude following occlusion in the ipsilateral hemisphere. One hour and 24 hours after embolization, clinical observation showed persistent rotation to the right. This model of focal cerebral ischemia mimics, closely a clinical embolic stroke and could be developed into an awake stroke model.

Supported by NIH NINDS grants NS21538 and NS24343.


In several rodent models phenytoin (P) has been reported to reduce neuronal cell loss following global cerebral ischemia or permanent focal ischemia resulting from MCAO. We examined the distribution of P in brains of MCAO (with ipsilateral carotid artery occlusion) and sham operated animals. Animals were injected with 50mg/kg of P 45 min before or 30 min after surgery and sacrificed under halothane anesthesia at 0.25, 1, 2, or 4 hrs after dose for sectioning and quantitative autoradiographic analysis. The total uptake activity (R) was evenly distributed in gray matter at 15 min. although slightly lower levels were seen in neocortex on the operated side. The P uptake in the small small (2-4mm) area originating in agranular-parietal cortex contained significantly lower R than surrounding tissue, in spite of the fact that uptake was in separate studies of rats kept two days after MCAO much larger areas were affected. From 1 through 4 hours in R in this small area (core of infarct) increased to 2 fold. The uptake was considerably sur-
rounding cortex or sham operated cortex at 4 hr. Distribution patterns suggest diffusion of R into ischemic regions presumably from residual collateral circulation. Thus, P may result from lack of circulation in the area.
386.1

Possible gender-related differences in ischemic and post-ischemic pathophysiology were examined in Mongolian gerbils subjected to a 3 h period of unilateral carotid occlusion (UCO). Only gerbils showing neurological signs of UCO-induced cerebral ischemia were studied. In initial experiments, it was observed that females displayed significantly less 24 h post-ischemic brain damage, as measured by histological criteria, in the hippocampal CA1 and ipsilateral to the UCO occluded carotid regions. Males exhibited 34.6% more necrosis in CA1 and 38.3% more in the lateral cortex. In a second set of mechanistic experiments, no difference was observed in cortical blood flow between the two sexes before, during or for 2 hr after the 3 h UCO. Moreover, the ischemic decline in cortical extracellular calcium did not differ, but the post-ischemic recovery was significantly greater in females. Measurement of brain vitamin E levels as an index of lipid peroxidation (LP) showed a 43.5% decline by 2 hrs after reperfusion in males while in females the decline was only 4.2% (p<0.05). Previous studies showing protective effects of antioxidant in males have suggested an important role of oxygen radicals in LP. Thus, it is postulated that the lesser ischemic vulnerability and preservation of brain vitamin E in females may be due to an antioxidant effect of endogenous estradiol.

386.2
COUPLING OF METABOLISM AND ELECTRICAL ACTIVITY IN CORtical ASTROCYTES IN CULTURE. D.R. Harold and W. Heiz. Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask., SN OWO, Canada.

The effect of metabolic inhibitors and of ouabain on membrane potential and input resistance of primary cultures of astrocytes from newborn Swiss mice was evaluated. Astrocytes always react to metabolic inhibition with an immediate depolarization and not with hyperpolarizations as neurones. Antimycin A caused depolarizations of up to 50 mV. These depolarizations are always a 40% shift of the resting membrane potential. Thus astrocytes are well capable of surviving a prolonged hypoxic insult. The glycolytic inhibitors Na fluoride and iodoacetic acid caused an initial depolarization rate of 0.6 respective 0.7 mV/min, which is less than the 3.2 mV/min caused by ouabain. Addition of ouabain did not exceed the 1.2 mV/min rate. Thus it seems that inhibition of glycolysis exerts its effects by Na+, K+ pump blockade via ATP depletion. Long-term exposure (<20 min) to the reversible glycolytic inhibitor Na fluoride caused irreversible effects dependent on the amount of depolarization which were accompanied by a 3-5 times increase in resistance. Astrocytes were usually capable of recovering well from similar depolarizations caused by ouabain. Thus, we conclude that inhibition of glycolysis is causing irreversible damage, which is not directly related to the resulting ion gradient breakdown.

386.3

Production of hydroxyl free radicals is difficult to measure due to their short half-lives. We report here a method to measure MSA, the immediate and stable product of a trapping reaction between a hydroxyl radical and DMDSO. We used HPLC with dual-coulometric electrochemical detector. The column was silica-based triflamy (Burdock and Jackson, 5 micron) and the mobile phase (pH 3.4) was sodium acetate (100 mM) and tetrabutylammonium hydroxide (200 mM). Output was monitored from the second working electrode (W2). MSA produces a response approximately 30,000 times greater than DMDSO when allowed a greater amount of small amounts of MSA in the presence of large amounts of DMDSO, even though the resolution alpha between the two compounds is only 1.2. The detector response was linear for MSA between 0.39 and 200 pmol. This method was utilized to quantitate MSA in perfusate from the transcardiochelically perfused rat to study pulmonary generation of hydroxyl radicals during reperfusion injury. No sample preparation was required prior to the HPLC analyses. The within-assay coefficient of variation (CV) for these samples ranged from 0.40 to 1.44 percent (n=3); the between-assay CV was 5.4% (n=2).

386.4
BRAIN DAMAGE IN THE NEWBORN PIGLET AFTER HYPOXIA AND SEVERE PNEUMOTHORAX. C.S. Easley,* A.E. Kopelman,* F.S. Nusser,* T. Conner-Keretta,* of Pediatrics and Anatomy/Cell Biology, East Carolina University School of Medicine, Greenville, NC 27834.

We examined the effect of severe pneumothorax (SP) in combination with hypoxia on brain histopathology in 32 newborn piglets. This model parallels hypoxia/ischemia seen in newborn infants with air block syndrome. We randomly assigned the piglets to a group control, hypoxia only, SP only, or a combination of hypoxia and SP. We produced hypoxia in halothane-naive piglets by having them breathe a 1:1 mixture of air and nitrous oxide for 2 hours. We then induced SP by injecting air into the right pleural cavity until the mean systemic blood pressure fell to 33% of baseline and was maintained for 4-16 minutes. Blood pressure, heart rate, blood gases, and blood chemistries were monitored throughout the surgical procedures. We resolved the SP and re-stabilized and maintained the piglets for 3-7 days. The basal ganglia, neocortex, and hippocampus were examined for pathologic changes using a silver impregnation method highly selective for degenerating neurons. The brain regions were scored by an independent observer for the presence or absence of damage and analyzed using a Fisher's exact test. Damage was found only in the animals exposed to a combination of SP and hypoxia. Neurons containing silver precipitate were found in the dural lateral basal ganglia and the depths of neocortical sulci. We rarely found degenerating neurons in the hippocampus. (Partially supported by the NC Affiliate of the American Lung Association).
NEUROPROTECTIVE EFFECTS OF ADENOSINE AGONISTS ON CEREBRAL ISCHEMIA IN THE GERbil. R.L. Dean, D. Lutz, S. Mennerick and R.T. Badger. CORTEX Pharmaceuticals, Inc., Irvine, CA 92715, USA.

Bilateral occlusion of the carotid arteries in the gerbil (a model of cerebral ischemia in humans), followed by reperfusion, results in elevated glutamate levels which lead to hippocampal degeneration. Adenosine A1 receptors exist in areas particularly affected by ischemia (e.g., hippocampus, striatum, cerebral cortex). This distribution is similar to that of the glutamate receptor subtype, NMDA. Since adenosine analogs are potent inhibitors of the presynaptic release of glutamate and down-modulate the postsynaptic effects of NMDA stimulation, adenosine agonists may be useful for the treatment of ischemia and stroke.

This study was designed to determine: 1) the effect of adenosine as a neuroprotectant when compared with NMDA receptor antagonists, and 2) the neuroprotective role of A1 vs. A2 adenosine receptor-specific compounds. First, we directly compared the effects of pre-occlusion administration (90 min) of the adenosine analog, AZA, with the specific NMDA antagonist, CPP, and the non-competitive NMDA antagonist, MK-801, for their ability to protect both histologically and behaviorally against ischemia-induced CA1 neurodegeneration (6 minute occlusion). Next, the effects of a selective A1 agonist (CHL), A2 agonist (DMPA), and the mixed A1/A2 adenosine agonist (NECA) were directly compared to further characterize the neuroprotective role of adenosine in this model. Because adenosine agonists produce neuronal hyperpolarization, the peripherally vs. centrally mediated protective actions were also characterized and will be discussed.

ATP DOES NOT CORRELATE WITH RECOVERY FROM ANOXIA WITH THIOPENTAL IN THE RAT HIPPOCAMPAL SLICE. L.S. Kass, J.R. Cotrell* and G. Chambers* Anesthesiology Dept. State University of New York, Stony Brook, Stony Brook, NY 11794-8400

We used the in vitro hippocampus as a model system to examine whether CA1 pyramidal cells were protected against anoxic damage by thialopenal. The postsynaptic evoked population spike was recorded from the CA1 pyramidal cells after stimulation of the Schaeffer collaterals. Significance was determined using ANOVA and t-tests (p < 0.05; values are mean ± standard error). When the CA1 pyramidal cells were subjected to 3.5 min. of anoxia the postsynaptic population spike recovered to only 1024 % of its preanoxic amplitude. If slices were treated with thialopenal (600 µM) 15 min before and during anoxia there was 100% recovery of the response. Thus thialopenal significantly improved recovery of the response after anoxia. A lower concentration of thialopenal (250 µM) also showed significant protection (2423%), however there was a clear dose related effect. The lowest dose of thialopenal tested (100 µM) did not show significant protection (1725%). Thialopenal (600 µM) significantly decreased the level that ATP fell to in the CA1 region during 3.5 min of anoxia (2.3±1.1 nmol/mg dry wt, untreated; vs. 1.6±0.7(thialopenal). After 5 min of anoxia there was no significant difference between treated and untreated slices. Only after 10 min of anoxia did thialopenal show a slight preservation of ATP during anoxia.

Thus neurons in the rat hippocampal slice recover better from short periods of anoxia if they are treated with high concentrations of thialopenal but this protection does not correlate with ATP levels during anoxia.

FOCAL CEREBRAL ISCHEMIA: CHARACTERIZATION OF INFARCT EVOLUTION AND EFFECTS OF GMI TREATMENT. M.S. Seren, T. Koop, R. Schisavo, A. Lazzaro, G. Vantini, O. Toffano and A. Legn. Fidia Research Laboratories, 35031 Abano Terme, Italy

In the experimental model of rat middle cerebral artery occlusion (MCAO) according to Tewura, both cortex (mainly the parietal portion) and striatum are severely damaged. In these two areas we evaluated the temporal profile of infarct progression by assessment of water accumulation (edema), ionic alterations as well as loss of characteristic neurotransmitter-related parameters. Cerebral edema appeared already at 3 hrs after MCAO reaching its maximum at 24-72 hrs and thereafter declining. Calcium and sodium accumulation showed a similar trend. Furthermore, focal ischemia induced a severe and long-lasting loss of choline acetyl transferase (CHAT) activity as well as of dopamine content. These metabolic alterations were more pronounced in striatum than in cortex. Chemical alteration was associated with diffuse morphological damage which began to be histologically evident already after 2 hrs. In addition, the exogenous administration of dopamine (10 mg/kg) did not alter the evoked significant neuroprotective effect. These latter results strengthens the therapeutic potential of the ganglioside in clinical situations of cerebrovascular insufficiencies.
386.13 MEMBRANE FATTY ACID CHANGES IN PRIMARY & PERIPHERAL TISSUES AFTER ACUTE GMI GANGLIOSIDE TREATMENT. H. Hugend, V. Oskale, A. Ortiz, S. P. Karkip, and S. P. Mahdik, Div. Neuroscience, NYSPH and Columbia U., P.S. (P.A.S), N.Y., N.Y. We have shown that Ca⁺⁺ and Na⁺,K⁺-ATPase activity were significantly increased after cerebral ischemia (150min) in rats. Rats were sacrificed at 3-72hrs, 4&8hrs, & 8hrs in control and in both a primary & peripheral ischemia area. We report here an autoradiographic study in which membrane fatty acid (FA) changes were reduced with GMI ganglioside treatment. Here we report this protective effect of GMI in focal cerebral ischemia (permanent occlusion of the MCAO & ipsilateral common carotid artery (CCAO)) & the temporary controlateral CCAO). Three groups of rats were used - ischemic (GMI 10mg/kg, i.c.), ischemic saline (placebo), & sham. Rats were sacrificed at 3&72hrs, 4&8hrs. FA levels were significantly reduced in GMI treated rats compared to saline treated controls and were shown to be significantly increased in area B of the ischemic hemisphere compared to area A. FA levels were also significantly increased in the peri-infarct area in both groups. 3hrs, in all groups there were no changes in FA levels. In the controlateral tissue in all groups, FA levels were unchanged. The data indicate that GMI treatment restores membrane FA metabolism in ischemia. Supported in part by NINDS (NS-257865) & FIDIA Research Foundation.

386.15 GMI GANGLIOSIDE TREATMENT MAINTAINS CAPACITY OF ISCHEMIC TISSUE TO DEFEND AGAINST FREE RADICAL DAMAGE. S. P. Mahdik, J. Muzi, M. S. Muzi, and R. P. Karkip, Div. Neuroscience, NYSPH, and Columbia U., P.S. (P.A.S), N.Y., NY. We have shown that, in cerebral focal ischemia occurs a series of acute biochemical changes that appear to be characterized by levels of Na⁺, K⁺-ATPase activity. We have begun a study to examine the effects of GMI (10mg/kg, i.c.) on these parameters. GMI treated rats were treated with GMI immediately after ischemic damage. Enzymes were assayed at 4, 8, and 24hrs. No differences were found between GMI treated and saline injected ischemic rats. We have found that acute GMI ganglioside treatment of ischemia reduces membrane function losses. We have tested whether GMI increases enzyme levels of ischemia-induced metabolites. No changes were treated at 4, 8, and 24hrs. No differences were found between GMI treated and saline injected ischemic rats. We have reported that GMI increases enzyme levels of Na⁺, K⁺-ATPase activity. We have begun a study to examine the effects of GMI (10mg/kg, i.c.) on these parameters. GMI treated rats were treated with GMI immediately after ischemic damage. Enzymes were assayed at 4, 8, and 24hrs. No differences were found between GMI treated and saline injected ischemic rats. We have reported that GMI increases enzyme levels of Na⁺, K⁺-ATPase activity. We have begun a study to examine the effects of GMI (10mg/kg, i.c.) on these parameters. GMI treated rats were treated with GMI immediately after ischemic damage. Enzymes were assayed at 4, 8, and 24hrs. No differences were found between GMI treated and saline injected ischemic rats. We have reported that GMI increases enzyme levels of Na⁺, K⁺-ATPase activity.

Assessment of behavioral recovery of animals following focal cerebral ischemia is often difficult due to the subjective nature and limited scope of many behavioral tests. Using a number of behavioral tests, the present study quantified neuromotor status in rats with various focal cerebral middle cerebral artery occlusion (MCAO) and 6 sham operated rats. MCAO leads to reproducible infarction of the striatum and overlying cortex. Five tests designed to evaluate sensorimotor function and sensorimotor integration were given twice weekly for 1 week pre-operatively and 4 weeks post-operatively. ANOVA tests revealed a difference between groups pre-operatively. Two days after MCAO, infarcted animals displayed significant neurological deficits compared to controls in abnormal posture when lifted by their tails; in foot faults during the beam test and sensorimotor integration. In contralateral limb placing to visual and tactile stimuli. Observed deficits were maximal on days 2-7 post-operatively and gradually recovered to control levels by day 28. Deficits were not seen in ipsilateral limb placing or on a test of strength on an inclined screen. Following the 30 days of neurologic testing, animals were given 10 days of testing in the Morris water maze, a test of spatial mapping ability. While ANOVA revealed that both MCAO and sham groups showed a decrease in latency to locate the hidden platform over days, the MCAO animals were consistently slower (latency=16.8 ± 1.5 sec. vs. 6.3 ± 1.5 sec. on day 10) and significantly less accurate in their initial heading than were controls. This battery of tests reveals a comprehensive picture of the deficits following MCAO and indicates that while sensorimotor integration recovers over 28 days, cognitive mapping deficits persist, paralleling results reported in human patients with infratentorial ischemia.

ALZHEIMER'S DISEASE: CYTOSKELETON

387.1 TAU PHOSPHORYLATION IN HEAT-SHOCKED FEMALE AND MALE RATS AND CEREBRAL EXPLANTS. S. Ch. Papapolymerou and Y. St. Dept. Pathol., Univ. of Texas Med. Sch., Houston, TX 77225.

We have previously shown that in the somatodendritic compartment of neurons tau becomes phosphorylated at or near the epitope of the antibody Tau-1 preventing its binding. To investigate whether the excessive tau immunoreactivity seen histologically in Alzheimer's disease might be stress-induced, we have studied normal and heat-shocked (42°C for 15 min) 2-3-month old female (5 pairs) and male (8 pairs) Sprague-Dawley rats and control and heat-shocked (42°C for 20 min) fetal rat cerebral explants. Six hr after heat shock, immunoblots of cell extracts and explants were analyzed using Tau-1 (provided by Dr. L.I. Binder) and T-20 protein A with and without pretreatment with alkaline phosphatase. In heat-shocked female rats there was: a) excessive phosphorylation of existing tau and b) a new band of increased phosphorylation (ratio: phosphorylated + non-phosphorylated = total/non-phosphorylated Tau-1 epitope (0.64 ± 0.22, p < 0.0001); b) a significant increase in the total of total tau (cpxm-1591 ± 4.352, p < 0.05); and c) a marked reduction of non-phosphorylated taud (cpxm-1,661 ± 2.627, p < 0.01). Similar but less pronounced changes were observed in age-matched male rats with the respective numbers being: a) 2.78 ± 0.16 (p < 0.0001); b) cpxm-1593 ± 0.1736, p < 0.05; and c) cpxm-2,060 ± 3.585, p < 0.001. On the contrary, in heat-shocked cerebral explants there was a marked increase in the amount of total (cpxm-0,327 ± 0.443, p < 0.05) and (cpxm-5,455 ± 5.074, p < 0.001) tau and a pronounced decrease in the ratio total/non-phosphorylated (1.50 ± 3.12, p < 0.02) due to excessive amounts of non-phosphorylated tau. This decrease in phosphorylation caused loss of the normal slow moving 60 KD tau polypeptide. These findings suggest that tau plays a role(s) in the stress response by altering its state of phosphorylation.

387.2 ANTIGENIC HETEROGENEITY OF NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASE: W. Bondareff, C.M. Wischik, Dept. of Psychiatry, University of So. California, Los Angeles, and M.R.C. Laboratory of Molecular Biology, Cambridge, England.

Antibodies directed against three regions of tau have been used to distinguish two populations of neurofibrillary tangles, intracellular and extracellular. Using cross-species polyclonal antibodies directed against N- and C-termini of tau were shown to be antigenically distinct from intracellular to extracellular tangles; immunolabelled by monoclonal antibodies against antigens in the triple repeat region of the molecule. Exposure of the latter appeared to be associated with the proteolysis of paired helical filaments occurring in the extracellular compartment. In the CA1 region of hippocampus, a subset of extracellular tangles were immunolabelled also by monoclonal antibodies against T-amyl on in some patients. These patients were characterized by a severe degree of pyramidal cell loss and a high number of extracellular in CA1.

387.3 TRANSFORMATION OF cDNAs ENCODING DIFFERENT ISOFORMS OF HUMAN TAU. M.M.S. Loi, A.W. Fields*, C.B. Caputo*, L.G. Sygowski*, C.W. Scott, and G. Goedert*. 1ICIS America, Wilmington, DE 19897, and 2MRC Laboratory of Molecular Biology, Cambridge CB2 4HQ, UK.

Tau proteins are low molecular weight microtubule-associated proteins normally expressed throughout the mammalian nervous system. Tau is also found in the progressive core of the normal, helical filaments which forms the bulk of the neurofibrillary tangles found in neuropathologic brain samples of Alzheimer's disease. The genes encoding for six different tau isoforms, cloned from human cDNA libraries (Goedert et al., Neuron 3:519, 1989), were subcloned into expression plasmids and transfected into 3T3 fibroblasts and PC12 cells. High levels of trans_temp type 3 tau (repeat) expression was detected by immunofluorescence assays with anti-tau antibodies in 3T3 cells. In most cases tau protein was in either small intense patches within cell bodies or in processes. In one case intense nuclear staining was detected. Stable PC12 transformants, cotransfected with the neo gene and selected with G418, showed very low levels of tau expression. In contrast, moderate levels of stably transfected 3T3 cells. Tau staining was found in the cytoplasm and very long, thin, and branched "neurite-like" extensions. The stability of microtubules formed within these cell lines (and others transfected with plasmids encoding for the different human tau isoforms) will be tested by treatment with microtubule-depolymerization drugs such as colchicine and nocodazole.

387.4 ALZHEIMER BRAIN FRACTIONS BEARING PHF CONTAIN WHEAT GERM AGGLUTININ BINDING ACTIVITY. L. McGaughin, F. Zemlin, and G. E. Dean. Department of Mol. Genetics, Biochemistry, and Microbiology, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0524.

Alzheimer brain are transformed by paired helical filaments, composed primarily of paired helical filaments (PHF), have been previously reported (Szumanska et al., Proc. Nat. Acad. Sci., 71:31-11) to bind wheat germ agglutinin (WGA). Fractions of Alzheimer brain extracts highly enriched in PHF were fractionated by SDE PAGE, electroblotted, and the blots were probed with WGA coupled to horseradish peroxidase (HRP). Alzheimer-specific reactivity was observed at positions corresponding to Mr's of 85, 74, 39, and 37 KDa, lacking in similarly obtained fractions from normal brain. Extracts probed with antibodies reactive with purified PHF proteins, immuno-reactivity was observed at Mr positions corresponding to 85 and 37 KDa in both Alzheimer and normal samples, suggesting that certain normal proteins are inappropriately glycosylated in Alzheimer brain tissue.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

WEDNESDAY PM

ISCHEMIA VI
387.5 PHOSPHORYLATED TAU IN ALZHEIMER’S DISEASE PAIRED HELICAL FILAMENTS. L. Baum, E. Marilaj, K. Udeta1, D. Irimoto, T. Satch, UCSF, School of Medicine, Dept. of Neurosciences, M-024, La Jolla, CA 92039.

Paired helical filaments (PHFs) are found in the degenerating neurites surrounding amyloid plaques, and in the neurofibrillary tangles (NFT) of Alzheimer’s disease (AD). A major PHF constituent is abnormally phosphorylated tau, a microtubule-associated protein. Phosphorylation of microtubule-associated proteins is known to reduce their ability to promote microtubule polymerization. One could speculate that abnormal phosphorylation of tau may disrupt the neuronal cytoskeleton, contributing to cell injury and death.

Either alpha or alkaline phosphatase treatment of AD brain sections enhances NFT staining with anti-tau monoclonal antibody (mAb) Tau-1 suggesting that the Tau-1 epitope is masked by phosphorylation in AD NFT. Phosphatase treatment of immunoblots of AD brain pellet fractions and of purified PHF also enhanced Tau-1 binding to a double of ~60 kD mol wt.

NFT is also stained by Alz-50, a mAb that preferentially stains AD brain and is not sensitive to alkaline phosphatase treatment. However, we found that acid phosphatase reduces Alz-50 staining of NFT. Furthermore, phosphoserine, but not serine or phosphothreonine, diminishes Alz-50 staining of NFT, pointing to phosphorylated serine(s) on tau as the epitope for Alz-50.

The tau sequence contains at least four theoretical serine phosphorylation sites for casein kinase II (CK-II), and tau is phosphorylated by CKII. We previously found that anti-CKII immunoreactivity is localized to NFT, and that purified CKII binds NFT on AD brain sections. We are now investigating the possibility that CK-II may contribute to the phosphorylation of tau at Tau-1 or Alz-50 epitopes.

387.7 EXPRESSION OF PAIRED HELICAL FILAMENT ANTIGEN IN ALZHEIMER’S DISEASE AND ISCHEMIA. M.M. Robertson, N.P. Galletly, S. Greenberg, P. Davies and C.H. Saper. Deps. of Pharm. & Physical Sc., and Neurology, Univ. of Chicago, Chicago, IL 60637 and Dept. of Pathology, Albert Einstein Medical College, Bronx, NY 10461.

PHF-1 is a monoclonal antibody raised against a denatured preparation of paired helical filaments from brains of patients with Alzheimer’s disease (AD) (Greenberg and Schein, Soc. Neurosci. Abst. 15:87, 1989). It recognized PHF-1 immunoreactivity in the brains of six patients with AD, four controls without neurological disease and one 22-y.o. patient with a transient global ischemic event. In the normal controls, PHF-1 stained mainly in a fibrillar pattern. In the AD brains, there was fibrillar staining of cell bodies and associated dendrites, dystrophic axons in fiber pathways and dystrophic neurites in neurofibrillary plaques. The axonal and laminear staining pattern was similar to the AD pattern seen with another monoclonal antibody, AlZ-50, as well as the AD neurofibrillary degeneration visualized using thioflavin-S staining, in adjacent sections from the same brains. In the ischemic brain, PHF-1 stained neurons in a granular, cytoplasmic pattern in the medial temporal lobe only. The densest staining was in the CA1 region of the hippocampal formation, and occasional neurons in the entorhinal cortex appeared to contain immunoreactive tangles. Our observations suggest that PHF-1 stains an epitope present on paired helical filaments in AD that is also present on a protein expressed during cellular ischemic stress.

387.9 A PROGRESSIVE APPEARANCE OF A TAU-RELATED EPITOME IN NEURITES CHARACTERIZES THE EVOLUTION OF ALZHEIMER’S DISEASE PATHOLOGY. R. Mora4, C.H. Wischik2, H. Novak3, C. Miletic4, A.C. Collin1, 1 Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, H3G 1Y6, Canada; 2 Department of Psychiatry, Cambridge University, Hills Road, Cambridge, CB2 2QQ, England; 3 Slovak Academy of Sciences, Bratislava, Czechoslovakia; 4 Laboratory of Molecular Biology, Medical Research Council, Cambridge, CB2 2QH, England.

The paired helical filament core (PHFcore) present in Alzheimer’s disease (AD) brains, contains the microtubule binding domain (Wisniewski et al., J. Biol. Chem. 263 (1988)). Using the monoclonal antibody 6.423, which specifically recognizes this tau-related epitope of the PHFcore, we studied frontal cortex of AD cases with short and long disease history. In all cases, 6.423 lab recognized both intracellular and extracellular neurofibrillary tangles (NFTs). However, in cases with longstanding dementia, a pattern of immunoreactive (IR) thread-like structures was observed in addition to the NFTs. The appearance of such structures seemed to lag behind that of IR NFTs. The IR thread-like material may represent fragmentation of the tangle and/or aberrant sprouting of affected neurons and, therefore, reflect the progression of AD pathology.

(Supported by NRC Canada and Medcorp, Canada.)

387.8 A NOVEL NEUROFILAMENT KINASE. H.M. Roder* and V.M. Ingram. Dept. of Biology, Mass. Inst. of Tech, Cambridge, MA 02139.

The two heavier subunits of mammalian Neurofilaments (NF) (Nf 68, 160, 200) aggregate in the presence of proline-alanine-proline (PAP) sequence, which are extensively phosphorylated for as yet unknown reasons. We are purifying and characterizing a corresponding kinase from bovine brain, purified by conventional 32P- assays and a novel immunosassay. This kinase might act also on an identical site in tau protein, which seems to be phosphorylated and occurs in the carboxy-terminal portion of the molecule incorporated into the hard core of Paired Helical Filaments in Alzheimer’s Disease.

A novel immunosassay based on completely dephosphorylated bovine NF-triplet and monoclonal antibodies specific for the phosphorylated Lys-Ser-Pro-sequence paved the way to partially characterize and purify a kinase activity from an ammonium-sulfate fractionated bovine brain supernatant. The activity survives chromatography only on a few media in the presence of Mg ions and ATP. DEAE filtration suggests a Mr around 60K with a probable proteolytic fragment at 40K. All NF-subunits, native or dephosphorylated, are substrates to varying extents. At pH 7.0 and 3 g/ml of P, are incorporated into dephosphorylated NF 160 and 200 with shifts to higher apparent Mr (50S-PAGE) after 18 hrs of incubation. The properties of the activity, such as lack of association with cytoskeleton proteins, stability, inhibition by isonicotinic acid, and kinetic parameters, identify it as a new kinase.


Abnormal phosphorylation of the microtubule-associated protein tau in Alzheimer’s Disease (AD) may be caused by altered expression of calcium/calmodulin dependent protein kinase II (cam kinase II) (Cam K II).

We performed in situ hybridization studies in the human CNS to examine the expression of cam kinase II and tau mRNA in the hippocampus and other regions of AD brains. We examined the presence of neurofibrillary tangles and neuroplaque in AD. In isocortex, the laminar-specific pattern of neuronal hybridization (layers III, V-VI) was similar for both mRNAs. In some regions of association cortex, cam kinase II mRNA levels were higher than tau in layer II. In allocortex, layer II contained the highest abundance of both mRNAs. The hippocampal formation, amygdala, and nucleus basalis contained high levels of both mRNAs. In AD, areas which appear to contain viable neurons but had large numbers of plaques and tangles showed similar levels of both tau and cam kinase II mRNAs as in control brains. Future studies will examine the expression of different subtypes of these mRNAs.


High resolution transmission electron microscopy (TEM) has shown that neurofibrillary tangles (NFT) contain in addition to paired helical filaments (PHF) numerous 2.10 ± 0.2 nm filaments. These 2.10 ± 0.2 nm filaments are triple-stranded left-handed helical structures composed of three 1.00 ± 0.2 nm strands. These filament structures have been compared with some of our TEM findings and found to have the same structure as judged by high resolution TEM. The reported amino acid sequence of human and bovine tau have been computer processed to predict secondary structure. Within the constraints imposed by the images, the secondary structure models and other structural information have been used to calculate tau’s maximum and minimum length. This work suggests that each ~1.0 nm strand is a tau sequence and that the ~2.1 nm filament is composed of three tau sequences (taus). Bovine tau length measurements points out that each single tau filament is made up of a fully stretched tau monomer. These measurements indicate that tau forms long polymers, (taus). A inverse temperature transition has been found in the circular dichroism spectrum of tau indicating that its structure is less ordered below 20°C and more ordered at 37°C. This phenomena helps explain how tau can reassociate during hyperthermic conditions and phase separate into microtubules below 20°C. The inverse temperature transition is also found in the protein elasin which aggregates at body temperature. If tau produced in Alzheimer victims was more hydrophobic than normal tau, it could aggregate into tangles similar to the mechanism of elasin aggregation.
387.11
CHARACTERISTICS OF THE TAU THAT IS FOUND TO ALZHEIMER'S
Group, CIG Americas Inc., Wilmington, DE 19897.

Tau protein is known to be a component of Alzheimer's
paired helical filaments. This present study investigated the
structural characteristics of the tau isolated from
promane treated FFRs (Vichk et al., PNAS, 85, 4606, 1988).
Antibodies with unique epitopes I to the tubulin binding
region of tau failed to reacted to promote treated
PPs. Three of 4 antibodies to promote treated PPs
reacted only after formalic acid or guaisidine treatment of
FFRs. Two of these antibodies (751 & 795) reacted to tau
isolated from normal brain whereas the other 2 (423 & 728)
only recognized PPR-derived tau. The phosphate dependent
antibody SN134 also recognized tau from PPRs. The
C-terminus of tau was present after promote treatment
based upon reactivity of an antibody raised to a peptide
from the C terminus of tau. In conclusion, the C terminus
including the whole tubulin binding region appears to be
protected from proteolysis by an interaction with other
PPR components & may contain phosphorylated sites & other
modifications or conformations that are not always present
in tau from normal brains.

387.13
 ACTIONS OF CALCIUM ON THE CYTOSKELETON AND TAU IN
 CULTURED HUMAN CEREBRAL CORtical NeurONS.
M. F. Mattox, Q. Perry and M. G. Zieg*.
Center on Aging and Dept.
of Anatomy & Neurobiol., Univ. of Kentucky, Lexington, KY 40536.

Abnormalities in the neuronal cytoskeleton are hallmarks of an array of
neurodegenerative disorders (ND). Some of the changes observed in the
human brain (e.g., paired helical filaments [PHF] and neuritic plaques)
are not observed in laboratory specimens. We have previously
begun to directly examine cultured fetal human cortical neurons
(see Rychik et al., this meeting). Since considerable circumstantial
evidence supports the involvement of altered cytoskeletal homeostasis in NDs
(Mech. Aging Dev. 50:103), we studied cytoskeletal changes associated
with calcium influx using immunocytochemistry and electron microscopy.
Axons in untreated neurons were dominated by parallel arrays of
microtubules. Exposure to calcium ionophore A23187 resulted in a
dramatic loss of microtubules, accumulations of microfilaments, and the
appearance of 12-15 nm straight filaments. PHF-like structures were
occasionally observed. Tau immunoreactivity increased in neurons
exposed to A23187, but Western blots indicated that tau levels (as well as
actin and tubulin) actually decreased as a result of calcium influx.
Colchicine (but not cytochalasin D) also increased tau immunoreactivity,
as a calcium-independent mechanism, suggesting that depolymerization of
microtubules may be the trigger for the altered disposition of tau, and its
accumulation in the straight filaments and PHF that characterize
neurofibrillary tangles. Immunoelectron microscopy is now being used to
more directly determine the fate of tau consequent to calcium influx.
(supported by a PSP grant and an ADRA Faculty Scholar Award to MM).

388.1
 FADING OF RECURRENt INHIBITION IN THE RAT DENTATE GYRUS
 IS MEDiated by GABA ReCEPTors. David D. Mott and Darrell V.
Lewis, Dept. of Pharmacology, Pediatrics (Neurology) and Neurobiology, Duke
University Medical Center, Durham, N.C. 27710.

Recent studies have shown that the GABA_B receptor agonist, baclofen,
suppresses recurrent inhibition in the dentate gyrus and facilitates the
development of long-term potentiation. Here, we present evidence showing that
released GABA, acting on GABA_B receptors, causes fading of recurrent
inhibition.

Fading of inhibition was studied in the dentate gyrus of the rat
hippocampal slice. Stimulation was delivered to the mossy fibers (MF) in
stream laminar CA3b and to the perforant path (PP) in the white spot
on the dorsal side of the hippocampal fissure. PP evoked a population spike (PS)
suppressed on a population epp recorded extracellularly in the
granule cell layer. MF stimulation inhibited a subsequent PP-evoked PS through
GABA_B receptor-mediated recurrent inhibition. A single conditioning MF
stimulus depressed subsequent recurrent inhibition. This depression of
inhibition was maximal 200 ms after the MF conditioning stimulus and lasted for
up to 2 sec. The GABA_B receptor antagonist, picrotoxin (1 mM) or 2-OH
saccharin (400 uM), abolished this depression of inhibition.

Intracellular recordings from granule cells demonstrated that MF
stimulation evoked a biphasic IPSP with an early component sensitive to
bicuculline (50 uM) and a late component sensitive to 2-OH saccharin (400 uM).
Paired MF stimulation caused a depression of the IPSP evoked by the second
stimulus. This depression was strongest when the interstimulus interval was 200 ms
and was antagonized by 2-OH saccharin (400 uM).

We suggest that GABA, released by the conditioning stimulus, acts on
GABA_B receptors causing a depression of recurrent inhibition.
Supported by NIH grant NS22170 and CIIT.

388.2
 AN AMPA/KAINATE ReCEPTOR-MEDIATED COMPONENT TO
 EpiLEPTiFOrM ACTIVITY IN THE KAINiC ACiD
LESioned HIPPOCAMPUS. L. Simpson* and H.V. Wheal,
Department of Neurophysiology, University of Southampton,
Bassett Crescent East, Southampton, S09 7TU, UK.

(Sponsored by the Brain Research Association, UK.)

In previous electrophysiological studies of synaptic activity in the
kainic acid-lesioned rat hippocampus, we have demonstrated graded
epileptiform bursting activity that is expressed in at least 50% of
The purpose of the present study was to further elucidate the
differences in the contribution of AMPA/kainate-receptors to suprathreshold
bursting activity in this model. Both intra- and extracellular studies were performed in the CA1 cells of lesioned hippocampal slices.

The AMPA/kainate receptor antagonist, CNQX (2mM), completely
blocked the evoked action potential in CA1 pyramidal cells in control slices. In contrast, in experiments on lesioned slices, CNQX failed to
abolish the evoked action potential. This outcome suggests that AMPA/kainate receptors contribute to the suprathreshold epileptiform burst in the
kainic acid lesioned hippocampus.
Supported by the Wellcome Trust.

Organotypic cultures of hippocampus (HPC) provide an excellent in vitro system in which structure and intrinsic connectivity are relatively preserved. Many reports of neuronal activity in these cultures document more epileptiform activity than acute adult HPC slices. To study the excitability of populations of HPC neurons, we used extracellular field recording technique to characterize the organotypic HPC culture electrophysiologically.

Organotypic slices were prepared from 6- to 7-day old HPC according to the methods of Gähwiler. Recordings were made in cultures of 5-5 DIV in a subculture chamber at 36°C with ACSF (95% O2, 5% CO2) perfused at 2 min. intervals. Extracellular field recordings were made on the pyramidal layer in CA1, with stimulation of Schaffer collaterals in CA3. Spontaneous interictal activity was uncommonly observed (<15% of cultures) and spontaneous ictal events were never observed. Evoked field responses showed EPSP with orthodromic population spike (PS) which was often followed by a burst of low amplitude PSs. Bath-applied 50 μM D-APV suppressed the late component of the EPSP and the superimposed bursts. Addition of up to 20 μM CNQX suppressed the remaining field EPSP and PS. Removal of Mg2+ from the bath induced evoked and spontaneous epileptiform activity (interictal and/or ictal) in CA1 which was also suppressed by 50 μM D-APV.

This method of slice culture provides a network virtually devoid of spontaneous epileptiform activity, yet this activity is readily inducible by removal of Mg2+. These electrophysiological features, together with the advantage of long term survival, make this culture a useful tool for studying the development of epileptiform activity in a simplified neural system.


Electrographic seizures (EGS) induced by stimulus trains in slices of adult rat hippocampus (HPC) have provided an acute in vitro model of epileptogenesis (Stahlheber et al, Science, 1989). Development of a chronic in vitro model would permit study of long-term plasticity in epileptogenesis. Organotypic cultures of HPC, with long survival time, may serve the purpose. We therefore sought to determine whether stimulus train could evoke EGS in this preparation.

Organotypic HPC cultures (12-16 DIV) were studied in a subculture chamber at 36°C with standard ACSF (95% O2, 5% CO2) bath. Extracellular field recordings were made in CA1 pyramidal layer, with stimulation of Schaffer collateral in CA3. One orthodromic responses to single shocks were stable, 2 sec trains of 60 Hz rectangular pulses (150-140 μA) were delivered every 10 min.

Stimulus trains reliably induced EGS with the typical pattern of initial high frequency (15 Hz) discharge, followed by slower frequency bursts (3-5 Hz). Induced EGS were of relatively long duration (5-19 sec), even on the first stimulation. With repeated stimulation, however, the duration of EGS did not reliably increase. Application of the NMDA receptor antagonist, D-APV (50 μM), modified the pattern of the EGS without consistently altering its duration. Even when D-APV was applied prior to the first stimulus, fully developed EGS appeared.

The reliable induction of EGS by stimulus trains offers considerable promise for development of a chronic in vitro model of epileptogenesis. The occurrence of a fully developed EGS on the first stimulus and the relative insensitivity of D-APV suggest that this preparation is hyperexcitability prior to stimulation. Study of various factors regulating this hyperexcitability during culturing process may shed light on mechanisms of epileptogenesis and on the ongoing plasticity in culture.

388.5 THE ROLES OF EXCITATORY AMINO ACID RECEPTORS IN EPILEPTIFORM ACTIVITY INDUCED BY 4-AMINOHIPRIDINE IN RAT AMYGDALA SLICES. F.W. Gean, Dept. of Pharmacology, College of Med. National Cheng Kung Univ. Tainan City, Taiwan, R.O.C.

The effects of excitatory amino acid receptor antagonists on epileptiform activity induced by 4-aminopyridine (4-AP) were studied in rat amygdala slices using intracellular recording technique. Five to ten minutes after switching to 4-AP-containing solution, spontaneous epileptiform bursts were observed in 35 out of 45 slices studied. Superfusion with kynurenic acid reversibly reduced the duration of the spontaneous bursting discharges in a dose-dependent manner. The IC50, estimated from the graph of the concentration-response relationship, was approximately 130 μM. In addition, CNQX which is a specific N-methyl-D-aspartate (NMDA) receptor antagonist blocked the spontaneous and evoked epileptiform bursting. In 11 out of 15 neurons tested, there was a residual depolarizing component remained. This depolarizing component was reversibly blocked by specific NMDA receptor antagonist, DL-2-amino-5-phosphonovaleric acid (DL-APV). Relative to CNQX-sensitive component, the DL-APV-sensitive component is much smaller in amplitude and shorter in duration indicating that NMDA receptor plays only a minor role in this process. These data suggest that the generation or propagation of spontaneous epileptiform events induced by 4-AP in the neurons of basolateral amygdala is mediated by excitatory amino acids and that activation of non-NMDA receptors is of primary importance.


Sprouting of the mossy fibers (MFs) into the inner molecular layer of the dentate gyrus of kainate-treated rats may cause formation of a recurrent excitatory circuit, which could hypothetically cause epileptogenesis. Extracellular recordings from slices of kainate-treated rats suggested hyperexcitability to hilar stimulation in normal solution (Tauc, D.L., and Nadler, J. Am. Acad. Neuro. 51:106, 1985). We have used simultaneous intracellular and extracellular recording in hippocampal slices to examine the electrophysiology of dentate granule cells that have undergone MF sprouting. In normal solution with MF sprouting usually had antidromic and synaptic responses to electrical stimulation of the hilus and perifornical path that were similar to those from control animals. However, when inhibition was reduced with the GABAa receptor antagonist, bicuculline, low-intensity hilar stimulation evoked delayed bursts of action potentials. Spontaneous bursts of population spikes, which were synchronous with intrahippocampal action potentials, were blocked by a depolarization, also occurred in bicuculline-treated slices. The delayed bursts to weak hilar stimulation and the spontaneous bursts in bicuculline were not observed in slices from kainate-treated rats without MF sprouting or in control animals. Thus, MF sprouting into the inner molecular layer of dentate granule cells can contribute to epileptiform activity when synaptic inhibition is reduced.

388.7 SYNCHRONOUS ACTIVITY INDUCED BY 4-AMINOHIPRIDINE (4AP) IN THE CA3 SUBFIELD OF THE RAT IMMATURE HIPPOCAMPUS. M. Avoli, V. Tancredi, C. Zona, and V. Putera. MNI and McGill Univ., Montreal, QC, Canada.

Field potential recordings were made in the CA3 area of hippocampal slices obtained from 10 to 30-day-old rats during perfusion with medium containing the convulsant 4AP (50 μM). Three types of spontaneous potentials were observed. The first one was a 4-11 s long potential (frequency: 20-100 Hz) that resembled an epileptiform interictal event. The second type was reminiscent of an ictal epileptiform discharge, lasted 10-35 s and was observed every 40-100 s. The third potential was of opposite polarity to the other two, occurred every 10-100 s and was usually followed by the ictal discharge. The occurrence of 4AP-induced antagonists did not modify any of the three events while the non-NMDA receptors antagonist CNQX blocked both interictal and ictal discharges. Thus, in addition to ictal activity of interictal and ictal type that is dependent on NMDA conductances, 4AP also induces the immature rat hippocampus synchronous event that is due to activation of the GABAa receptor.

Supported by The Hospital for Sick Children Foundation, the Savoy Foundation and the MRC of Canada (MA-8109).

388.8 NMDA-ACTIVATED CONDUCTANCES AND EPILEPTIFORM ACTIVITY INDUCED BY REPEETITIVE STIMULI IN THE CA3 SUBFIELD OF RAT IMMATURE HIPPOCAMPUS PERFUSED WITH 4-AMINOHIPRIDINE (4AP). V. Tancredi, C. Zona, and M. Avoli. Univ "Tor Vergata", Rome, Italy; MNI and McGill Univ, Montreal, QC, Canada.

When 4AP (50 μM) is applied to hippocampal slices obtained from 2- to 10-day-old rats, spontaneously occurring epileptiform activity is rarely seen in the CA3 subfield. In this experimental condition however, a short train of orthodromic spikes elicits a pharmacogeographic seizure (20-40 s) that is then followed by spontaneous epileptiform events that recur regularly for several tens of minutes (Tauc, D.L., and Nadler, J. Am. Acad. Neuro. 51:106, 1985). Any further electrical stimulus. This type of epileptiform activity is not influenced by the subsequent application of the NMDA receptor antagonist, CPP while being blocked by CNQX. Perfusion of non-stimulated hippocampal slices with medium containing CPP and 4AP does not affect the train-induced electrophographic seizures while being blocked by CNQX, but prevents the appearance of the spontaneous epileptiform discharges. Thus at this stage of brain maturation, NMDA-receptor antagonists are of critical importance in the generation of 4AP-induced epileptiform activity while being important for establishing the long-lasting state of hyperexcitability, which is associated with the occurrence of spontaneous epileptiform discharges. Supported by The Hospital for Sick Children Foundation, the Savoy Foundation and the MRC of Canada (MA-8109).
Epilepsy: Society

Electrographic Sprague-Dawley slices, but (N=7). We assessed the development of Na,K-ATPase activity in CA3 hippocampal neurons from slices of postnatal day (P) 7 to P39 rats. Spike amplitudes for neurons from P7-11, P21-25 and P35-39 age groups were 65.1 ± 11.1, 83.5 ± 7.6 and 84.3 ± 8.0 mV, respectively; resting potentials were 71.9 ± 0.6, 64.0 ± 6.6 and -63.7 ± 4.7 mV, and input resistances (R_i) were 50.2 ± 3.1, 36.4 ± 8.5 and 31.2 ± 8.5 MΩ. Similar glutamate-induced excitations (GiDs) could be observed in all three age groups. The size (area) of PGH was directly proportional to the area of the preceding GD. A ratio of PGH area to GD area was calculated for each neuron and used to evaluate Na,K-ATPase activity at different ages. The PGF ratios at ages P7-11, P21-25 and P35-39 were 0.82 ± 0.42 (n = 16) and 1.46 ± 1.00 (n = 5), respectively. However, in the P7-11 age group, PGF ratio was 0.30 ± 0.30 (n = 7), or significantly lower (p<0.01) when compared to the P21-25 value. Differences in membrane properties for these age groups do not explain the differences in PGF ratio. If anything, the higher R_i would tend to increase the sizes of PGHs in immature neurons. These results suggest that substantial Na,K-ATPase activity develops after birth. Low levels of electrogenic Na pump activity at early stages of development may be one factor contributing to the increased susceptibility of immature hippocampal to ictal discharges associated with prolonged membrane depolarizations. Supported by NIH grants NS02151 and NS04577 from the NINDS.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990


We have reported that electrographic seizures (EGOs) occur in hippocampal slices in physiological (0.9 mM Mg) medium when slices are from young (22-32 day old) rats, but not adult (6-8 day old) rats (Stansfeld et al, Science, 245:648, 1989). We report here that EGOs also occur in slices from older rats in Mg-free medium. Hippocampal slices (265 x 150 x 3 mm) were prepared from 190-211 day old Sprague-Dawley rats. Extracellular recording and stimulation were performed in stratum pyramidale and radiatum of CA3, respectively. When slices were bathed in Mg-free medium (< 0.5 mM of the GABA_A antagonist baclofen, EGOs either occurred spontaneously or following 1-3 stimulus pulses (N = 7). These EGOs had the same tonic-clonic morphology and duration (mean ± s.e.m = 105 ± 29 sec) as the seizures from young rats in Mg-free medium. However, the number of afterdischarge (AD) bursts per EGO mean ± s.e.m = 46) was only about half that of young rats. Additionally, 5-200 mM of the NMDA antagonist 2-amino-5-phosphonovalerate (APV) to Mg-free + baclofen medium substantially reduced, but did not block, AD bursting following 120 pulse stimulations (N = 5). When slices from older rats were bathed in 0.9 mM Mg + baclofen following EGO induction in Mg-free medium, 120 pulse stimulations produced only 0.5 AD bursts, indicating strong EGO inhibition (N = 7).

These results suggest that the increased suppression of EGOs that occurs with aging is partially reversed by removing extracellular Mg. In slices from older rats, EGO inhibition could be due to Mg being more effective in stabilizing the membrane or decreasing transmitter release. Alternatively, NMDA antagonists do not block EGOs in slices from young rats in physiological medium (Stansfeld et al., indicating that more Mg is sufficient to generate EGOs in these slices. In slices from older rats, the non-NMDA-dependent mechanisms alone may not be sufficient to generate EGOs in physiological medium, but may do so when augmented with NMDA-dependent mechanisms in Mg-free medium.

An a gene-linked, voltage-dependent prolongation of network-driven paroxysmal depolarizing shifts (PDS) has been identified in vitro during 10 mM KCl-induced bursting in tectofugal (ft) and ventral (f) medial amygdala (PMD) neurons (mean PDS durations: ft/g 196±11, n=10; f/g 119±9, n=11). We have explored local circuit synaptic mechanisms in the CA3 region that might mediate this state-dependent inherited excitability defect. Blockade of GABA_A receptors with bath applied 40 μM (DL)APV produced approximately 30% reduction in PDS duration in both genotypes (ft/g 125±6.0; 13±5, n=5; f/g 84±11±1.1, n=5), but did not selectively correct the mutant phenotype, suggesting that increased synaptic excitation through NMDA receptors is unlikely to contribute to the altered network response. In contrast, blockade of GABA_A receptors by 50 μM picrotoxin significantly prolonged (85%) the mean PDS duration in ft/g neurons (217±8±35.5, n=4), but had no significant effect on the mutant PDS (173±2.0±25.0, n=4). These data support the hypothesis that the prolonged PDS in ft/g CA3 pyramidal neurons may reflect an underlying attenuation of GABA_A-receptor mediated synaptic inhibition. Since intracellular recordings in ft/g CA3 cells under physiological 3 mM K+ conditions clearly show spontaneous and evoked inhibitory synaptic potentials, this evidence raises the possibility that the ft/g mutation interacts with mechanisms regulating activity-dependent modifications in synaptic inhibition. (Supported by NIH and Pew Foundation (JLM)).
EPILEPSY: BASIC MECHANISMS II
WEDNESDAY PM

388.15 CHRONIC LESION OF MEDIAL SEPTAL NUCLEI INDUCES AN EPIL-
ET-PATTERNED ACTIVITY IN RAT HIPPOCAMPAL SLCSS. F.Berton*, R.
Behnichi, W.Francesconi*, B.Sidorov** & M.Brunelli. Dpt. Physiol. & Biochim. Univ. of Pisa,56100 Pisa, Italy
**Inst. of Higher Nervous Activity & Neurophysiol., Acad. of Sci. USSR, Moscow.

In the rat, hippocampal formation receives a well character-
ized septal projection from cholinergic and GABAergic neu-
rons located in Medial Septal nuclei (MS) and vertical limb of Diagonal band. The mechanisms underlying the effects of
septo-hippocampal fibres degeneration on the excitability of
the hippocampal neurons have not been carefully analy-
zed. Spontaneous and evoked multiple population spikes
(MPSs) were recorded in CA3 and CA1 regions in slices iso-
lated from animals sacrificed 1,2 or 4 weeks after electro-
chemical or chemical (local application of ibotenic acid)le-
sion of MS. Spontaneous and evoked CA1-MPSs disappeared
after cutting between CA3 and CA1 regions. In slices from
unlesioned or sham-operated rats we never observed such
an abnormal activity. In conclusion, septo-hippocampal fibres
degeneration induces an epileptiform activity in CA3 spread-
ing to CA1 region. Intracellular recordings from CA3 and
CA1 neurons are in progress.

388.16 INFLUENCE OF ADENOSINE TRANSPORT AND ADENOSINE
DEAMINASE INHIBITORS ON BICUCULLINE METHODICALLY-
INDUCED SEIZURES IN RAT PREPITIFORM CORTEX. G.Zhang, P.H.
Franklin and T.F. Murray. College of Pharmacy, Oregon State University,
Corvallis, OR 97331.
The results of our previous studies suggest that an adenosine receptor
population in rat prepitiform cortex (PPC) plays an important role in the
suppression of bicuculline induced seizures (Franklin, P.H. et. al., J.Pharmacol. Exp. Ther. 251:1229,1989; Zhang, G. et. al.,
Neurosci. Lett., in press). In the present study we evaluated the manipulation of
endogenous adenosine in this brain area as a strategy to effect seizure
suppression. All compounds used in these experiments were unilaterally
microinjected into the PPC. It was found that the co-administration of a
subconvulsant dose (1.6 mmoles) of the adenosine antagonist 8p-
sulfophenyl)theophylline (8mSP) with BMT (30 mmoles) markedly
increased the severity of BMT seizures. Dilaipeg an adenosine transport inhibitor, provided a
potent protection against BMT seizures with an E<sub>D50</sub> value of 5.6±1.6 mmol/rat.
Together, the proconvulsant effect of 8mSP and anticonvulsant action of dilaipeg indicate that endogenous adenosine in the PPC exerts an inhibitory tone which
affects seizure susceptibility. Furthermore, we found that exogenous adenosine
also afforded a dose-dependent suppression of BMT-induced seizures (E<sub>D50</sub> = 6.8±1.6 mmol/rat) in the PPC, and these anticonvulsant effects of
adenosine were significantly potentiated by the adenosine transport blockers
dilaipeg (0.5 mmoles) and nitrobenzylthionine phosphate (20 mmoles). Similarly an adenosine deaminase inhibitor 2-deoxycoformycin (11.2 mmoles)
produced a 2.9 fold potentiation of the anticonvulsant effect of adenosine. These findings therefore
imply that adenosine is a major modulator of neuronal activity in the PPC and the
inhibition of the adenosine transport system and/or adenosine deaminase influences extracellular adenosine levels in this paleocortical brain area.
(Supported by UPHS Grant NS23277).

388.17 IN VITRO AND IN VITRO ASSESSMENT OF NEURO-
TRANSMITTER AMINO ACID FUNCTION IN TETANUS
TOXIN INDUCED CHRONIC SEIZURE FOCI IN RAT
HIPPOCAMPUSS. C.M. Forcetbi, B. Lebeta* and D. Garant*. Dept Neurological Sciences, Rush
Medical College, Chicago IL 60612.
Tetanus toxin or vehicle was injected uni-
laterally into dorsal hippocampus of rats
with chronic depth electrode/guide cannula
implants. After injection, EEG and focal
in vivo micro-dialysis were performed in the
unanesthetized rats, either before seizure
onset, during the peak of limbic seizures,
or after seizure remission. Enriched synapto-
sonal fractions were prepared from hippocampi
dissected from these rats, and amino acid concentrations were assayed by reverse-phase
HPLC.
As expected, in tetanus toxin seizure foci
in vivo, K<sup>+</sup>-induced GABA release appeared
decreased, however synaptosomal GABA content
was remarkably consistent through the seizure
course and between toxin and vehicle groups.
This suggests that tetanus toxin inhibits
GABA release, but neither this effect nor the
subsequent seizures alter nerve terminal GABA
content.

388.18 OUABAIN-BINDING SITES AND mRNAs OF Na-K-
ATPases ARE DECREASED IN HIPPOCAMPAL AREA
CA1 OF RAT BRAIN AFTER KAINATE LESIONS OF
CA3. A.A. Maki J.I. Staley*, W.C. Brown, Jr. Medical Center and
Administration of kainate to rats causes loss of neurons of the hippo-
campal CA3 region and leads to a hyperexcitable state in CA1 pyramidal
neurons. We hypothesize that changes in ion homeostasis, especially
due to decreased expression of the Na-K-ATPase, may contribute to the
mod-ified properties of CA1 neurons. We used ouabain binding to
measure the number of Na-K-ATPase sites in CA1 AND CA3 areas
mRNA for Na-K-ATPase subunits. Both high and low affinity
[3H]ouabain binding sites were found in control and lesioned
hippocampus with K<sub>d</sub> values of 60-77 nM(high) and 960-1382 nM
(low). No differences were found in K<sub>d</sub> values between control
and lesioned animals. Numbers of low affinity binding sites differed only in CA3 where nearly complete destruction of pyramidal cells was observed. High affinity sites were decreased in CA1 as follows: stratum
oriens, 20%; stratum radiatum, 25%; pyramidal cell layer, 33%. In
addition significant loss of mRNA encoding for a2 and a3 isoforms of
the catalytic subunit of the Na-K-ATPase was found in CA1 pyramidal
cells. Since Na-K-ATPases containing a2 and a3 are known to bind
ouabain with high affinity, the in situ hybridization studies support the
ouabain-binding results. We conclude that the number of Na-K-
ATPase molecules with high affinity for ouabain in CA1 pyramidal
cells is significantly reduced and may lead to modified ion homeostasis in this
model of epilepsy. (Supported by NS 20452 and the VA.)

388.19 MODIFICATION OF HYPEREXCITABILITY IN THE SUBCORTICALLY
DENERVATED HIPPOCAMPUSS BY SEPTAL GRAFTS.
M. Hsu, Z. Horvath*, E. H. Gage, C. Slamka* and G. Buzsaki.
Department of Neurosciences, UCSD, La Jolla, CA 92093.

Suspension grafts of basal forebrain cholinergic neurons were
implanted into the hippocampus 3 months after limbic
(FF) transection and the emergence of hyperexcitability
(Deoscri.: 28:327,1989) was tested 9 months after grafting. The
threshold for evoking afterdischarges was significantly
elevated in the grafted rats relative to FF-only animals. The
correlation between perseverant path evoked extracellular PSP
and population spike was positive in unoperated control (UC)
rats and grafted rats but negative in FF-only rats. The
behavior-dependence (walk vs. still) of pop-spike amplitude
was larger in the FF group, and smallest in the UC animals with
the grafted rats intermediae. Behavior-dependence of PSP was
similarly larger in FF and grafted groups relative to UC rats.
Long-term potentiation (LTP) of the dentate population spike
damaged was impaired in the FF-only rats; grafting did not result in
significant improvement of LTP. High frequency (18-32/sec)
power in the hilus was significantly larger during waking
than during immobility in all groups, and behavioral
modulation was significantly larger in the grafted animals and
FF rats than in the UC group. We conclude that delayed grafting
of cholinergic cells is capable of modifying the already
established hyperexcitability patterns of the denervated
hippocampus, although not all changes are restorative.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
389.1
ABNORMAL LYSOSOMAL HYDROLASE DISTRIBUTIONS IN ALZHEIMER BRAIN. A. M. Cataldo*, A. Pope, and R. A. Nixon. McLean Hospital, Harvard Medical School, Belmont, MA 02178

Lysosomal proteins in enzymatically active form are abnormally present extracellularly in classical scale plaques (SP) (Cataldo and Nixon, PNAS). We have proposed that these aberrant lysosomal enzymes are the principal source of these enzymes in SP and that the unregulated action of lysosomal hydrolases leads to abnormal metabolism of the amyloid precursor protein (APP). To further establish this idea, we localized other lysosomal hydrolases in Alzheimer brain. Sections of neocortex from control and AD brains were analyzed by immunocytochemistry with polyclonal antisera to the cat, protease enzymes, \( \alpha \)-galactosidase (GAL), \( \alpha \)-hexosaminidase A (\( \alpha \) HEX A), \( \alpha \)-glucosidase (GLU) and by enzyme cytochemistry for aryl sulfatase and acid phosphatase activities. Scale plaques were also identified histologically using Bischleowski silver stain and thioflavin S histochemistry. HEX A, GAL and GLU were present in neurons of control and AD brains and were abundant in senile plaques of AD brain. In SP, these hydrolases were localized by immunoelectron microscopy to extracellular membrane-limited profiles. Neurofibrillary tangles were also intensely stained with GAL antiserum. The activities of aryl sulfatase and acid phosphatase were detected in many SP even though activities in neurons were below the sensitivity limit of the method. The abnormal localization of various lyosomal hydrolases with extracellular \( \beta \) amyloid deposits supports a model involving neuronal degeneration and persistence of the lysosomal compartments which are capable of unregulated proteolysis including possible abnormal breakdown of APP. The aberrant trafficking and release of membrane-degrading hydrolases may be one mechanism to expose the intramembrane A4 cleavage site.

389.2
COMPLEMENT PROTEINS ARE ASSOCIATED WITH MEMBRANE ATTACK IN ALZHEIMER DISEASE. P. L. McGreer, H. Akiyama, T. Kawatama*, E. G. McGeer, K. Kineman of Neurology, UBC, Vancouver, BC, Canada, V6T 1W5

The pattern of Alzheimer tissue staining by antibodies to complement related proteins indicates that this activity as well as tissue destruction may be taking place. Extracellular debris in the form of amyloid deposits and ghost tangles are stained by antibodies to the complement components C1q, C4 and C5. The EP42835F antibody is able to stain these tangles. This pattern of staining suggests that the complement system may be involved in the pathogenesis of Alzheimer disease. Evidence for the activation of the complement system in Alzheimer disease includes the presence of possible MAC insertsions in cell membranes, depleted neuronal elements, and microglia in the CSF-9 complex. Many of these tangles and dystrophic neurites are also stained by an antibody to the membrane inhibitor of reactive lysosomes (MIPL). MIPL was shown to be localized in cell membranes, neuronal and microglial elements in Alzheimer disease. This data suggest that the complement system is involved in the neuronal and microglial elements in Alzheimer disease.

389.3

The critical diagnostic issue for Alzheimer disease (AD) is regional quantification of pathology in vivo. Magnetic resonance imaging (MRI), capable of measuring a variety of cerebral characteristics with considerable dynamic range, might achieve this quantitation. For example, senile plaques (60 \( \mu \)m dia., about 10 mm\(^2\)) at diagnostic threshold (% of tissue volume) might have a property which can be assayed via signal intensity changes (T1=40 ms, T2/T1=2, properties not distinguishing gray and white matter/CSF).

This protocol, statistical enhancement (fig. 1) of coronal images (fig. 2) showed a pattern of intensities in 6 probable AD patients suggestive of the distribution of AD pathology (data shown from autopsy-confirmed AD patient).

389.5

Previous studies have reported signs of nuclear atrophy in Alzheimer's disease, associated with loss of cholinergic markers in the cortex. It has been suggested that this might be partially responsible for the cognitive decline associated with the disease. We have demonstrated a relationship between normal age and Alzheimer's disease, we have determined the density of neurons, tangles, and plaques/normal and immunoreactive for the Alz-50 antibody and an antibody against paired helical filaments (donated by Dr. Peter Davies and Dennis Selkoe) in the cell clusters of the NBM. The cases studied were evaluated in the Alzheimer's Disease Research Center of Washington University and assigned a Clinical Dementia Rating (on-demented: CDR=0, very mildly demented: CDR=0.5, demented: CDR=1.5).

The density of large neurons was determined at three levels of the NBM. Four mildly demented cases (CDR=0.5 to 1) showed no loss of cells when compared to three non-demented cases (ca. 65 cells/mm\(^2\)). However, three more severely demented cases (CDR=2 to 3) had much lower cell density (ca. 20 cells/mm\(^2\)). The density of tangles and immunoreactive cells in the NBM in the mildly demented cases was slightly above that seen in non-demented elderly cases (average tangle density 2/mm\(^2\)). The density of these markers was greater in the severely demented cases (6/mm\(^2\)), but is much less than that in the hippocampal formation or neocortex. These observations indicate that cellular damage in the NBM is not a substantial feature of the early stages of Alzheimer's disease; it might contribute to the cognitive loss in later stages of the disease, but in such cases there is also substantial damage to the hippocampus and neocortex. Supported by NIH grants AG03991 and AG05811.

389.4

We used immunocytochemistry in order to examine the morphologic features of three neuropeptides (somatostatin (SOM), vasoactive intestinal peptide (VIP), and neuropeptide Y (NPY)) and of choline acetyltransferase (ChAT) in the amygdala of patients with AD and in 2 groups of controls. One control group (normal) had few if any plaques, while the other group (high N" plaques) had numerous plaques. The topography of the pathologic lesion was determined using Thioflavine-S. In AD patients and the high N" controls, immunolabeling for ChAT and NT was reduced in density relative to normal controls, while SOM and VIP labeling were normal in both groups. The reductions in NT and ChAT immunoreactivity were more pronounced in the AD cases than in the high "N" cases. Despite these differences in density, immunoreactive fibers of all four neuropeptides showed similar morphologic alterations in both the AD cases and the high "N" cases but these were not observed in the control brains. These alterations were characterized by gross vacuole swellings which were often associated with plaques and were most prevalent in areas of high plaque density. Most importantly, in the areas of highest plaque formation in the AD cases (which coincided with the areas of plaque formation in the high "N" cases), ChAT and NT processes were nearly completely absent and were rarely found within plaques, while in these same areas in the high "N" controls, ChAT and NT were present in reduced amounts and had abnormal fibers that were found within plaques. Abnormal SOM and Sub-P fibers were present within plaques in these areas in both the AD and the high "N" cases. The observed alterations in all four neuropeptides display similar morphologic irregularities suggesting a common pathological process. The reductions in ChAT and NT suggest that these transmitter systems may be more susceptible to the pathologic changes.

389.6
BASAL FOREBRAIN PROJECTIONS TO THE FRONTAL POLE OF THE THALAMIC RETICULAR NUCLEUS (MEDIODORSAL NUCLEUS AND FRONTAL CORTEX AREAS) IN THE OLD WORLD MONKEY, AND THEIR DISRUPTION IN ALZHEIMER'S DISEASE. W.G. Tourtellote, J.A. Blakley, and D.W. Van Hoomissen. Dept. of Anatomy and Neurology, Univ. of Iowa, Iowa City, IA 52242.

The thalamic reticular nucleus (RT) forms a narrow band of largely GABAergic neurons around the margins of the mediodorsal, centromedial, centromedial and cholinergic fibers from the basal forebrain (BF) and the pontomesencephalic reticular formation (PMR). Basal forebrain and PMR can disinhibit RT neurons and facilitate thalicthamic transmission. Immunocytochemistry with Alz-50 and tau antibodies in Alzheimer's disease (AD; N=15) but not control brains (N=8) revealed terminal-like immunostaining in the central pole of RT. Nerve growth factor receptor (NGFR) immunocytochemistry and NADPH-dihydropyrase (NADPH) histochemistry were used to demonstrate the distribution of cholinergic neurons in the septal-BF complex and PMR, respectively, in some of the AD and normal brains. There was a consistent depletion of neurons and numerous thin fibers, Alz-50 and tau positive neurons in BF but not in PMR. Old-World monkeys received isotropic injections with either PHAL or WGA-HRP in the BF and RT. BF terminal fields of RT demonstrated retrograde transport to the mediodorsal nucleus (MD), medial prefrontal and orbitofrontal cortex, while BF and thalamocortical fibers were located in intermediate and caudal locations. Isotopic injections with PHAL confined to the pole of RT revealed dense projections to MD. The data suggest that corticohalamic and thalamocortical fibers from BF and frontal cortex synapse in the pole of RT. They also demonstrate that the RT pole receives cholinergic projections from the BF and the PMR. Since neither the neurons in the PMR nor MD appeared consistently involved in AD, the changes observed in the RT may well arise from Alz-50 and tau positive neurons in the posterior and intermediate sectors of the BF. This may reflect a disruption of modulatory influences by the BF on MD-frontal lobe interactions in AD. (Supported by: 5732 GM07353, NS 14944, PO NS 16322.)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
389.7

LN-1 is a monoclonal (lgM) antibody specific for an untranslated region present on B lymphocytes but not T lymphocytes or histiocytes. A previous report suggested that LN-1 is specific for CN5 microglia. We find, however, that this antibody specifically labels CNS astrocytes with reactive morphology as found specifically in AD brain tissue—exhibit increased expression of LN-1.

Frontal cortex samples from 9 AD and 9 age-matched neurologically normal controls were obtained at autopsy (mean autopsy time of 4 ± 2 hours), cut into 1 cm blocks, fixed for 24 hours in paraformaldehyde, sectioned at 30 μm, and processed for immunohistochemistry. Additional material for immunohistochemistry was paraffin embedded after 10% or 20% formalin fixation for various times.

LN-1 immunoreactivity was observed on highly reactive astrocytes near the border of cortical layers I and II. The integrity of staining and the number of labelled cells were elevated in AD. LN-1 immunoreactivity in the other cortical layers was less obvious, the labeling being weaker and the morphology is considerably less reactive. LN-1 positive astrocytes, morphologically nonreactive, were diffuse in the white matter. The specificity of the LN-1 antibody for only astrocytes is confirmed by morphologic criteria and by double-labeling with the astrocyte specific marker GFAP.

Increased expression of the immune system associated antigen LN-1 by cortical astrocytes in AD is consistent with demonstrations by our laboratory and others that the pathogenesis of AD includes a chronic inflammatory response.

(Supported by NIA AG07367 to JR)

389.8
MORPHOLOGIC EVIDENCE OF A DYNAMIC RELATIONSHIP BETWEEN MICROGLIA AND SENILE PLAQUE AMYLoid in ALZHEIMER DISEASE (AD): L.S. Permutt, M.A. Myers, E. Beemsterboer, and J.S. Cohan, Uniformed Services University of Medicine and Dentistry, MD 20783.

Amyloid and mononuclear phagocytic cells are intimately associated in several amyloidoses. In AD, amyloid/microglial associations have been seen with light (LM) and electron microscopy (EM). With EM, bundles of amyloid from compact cores were found interdigitated with microglial cell membranes and within intracellular membranous structures. These cells were morphologically characterized as resting microglia—suggesting a secretory rather than phagocytic role.

The present study examined the relationship between microglial morphology and the pattern of amyloid deposition in AD. Microglial cells were labelled with the lectin Ricinus communis agglutinin-1 for LM and EM. With LM, dense amyloid cores (stained with thioflavine S) were always tightly encircled by clusters of highly ramified microglia. Larger, diffused amyloid plaques were associated with globular-shaped microglia with few if any processes. With EM, cytoplasm from labelled resting microglia was found interdigitated with amyloid bundles; intracellular membranous structures were also seen. Round, dense body-filled microglia were scattered around diffused amyloid accumulations.

These data suggest a dynamic pathogenic relationship, which may vary with the microglial functional state: ramified microglia may secrete while rounded microglia may phagocytose amyloid. Thus, compact rather than diffused amyloid accumulations may represent an earlier form of senile plaques.

389.9
THE BRAINSTEM IN ALZHEIMER'S DISEASE (AD): D. Doucette, T. Ducey, and A. B. Schelbel, UCLA Anatomy Department, Brain Research Institute and Mental Retardation Research Center, L.A., CA 90024.

The immunohistochemical labeling by antiserum to amyloid P component (AP-C) has been compared to that seen in brains from AD patients and control cases from normal elderly volunteers (age: 60-90). These studies were performed in an experimental laboratory setting.

Samples from different elderly volunteers were obtained from the right frontal lobe of patients diagnosed with Alzheimer's disease (AD) prior to the initiation of a venous catheter and pump assembly for the infusion of benzodiazepine as an experimental therapy. Samples were obtained from 6 different medical centers. The pathologists confirmed the AD cases (n = 26) as compared to a group of samples from normal age-matched autopsied controls (n = 21) and age-matched autopsied AD brains (n = 11). All samples were assayed for choline acetyltransferase (ChAT), acetylcholinesterase (AChE), binding to [3H]-quinuclidinyl benzilate (QNB) as an index of total muscarinic cholinergic binding and [3H]-pirepinephrine (PE) binding as an index of M1 receptor subtype binding. Half levels of ChAT activity were decreased to 36% of aged-matched controls, and the loss of activity correlated significantly with the mini-mental state examination, an index of global cognitive function. ChAT activity in AD brain cortex was further decreased compared to controls, indicating a continuous further decline through the course of the disease. AChE followed a similar, less dramatic decline. No differences were seen among the groups in the PE binding was significantly decreased in autopsied AD cases compared to biopsy cases or autopsied controls.

389.10
IMMUNOHISTOCHEMICAL ANALYSIS OF THE NUCLEUS BASALIS OF MEYNER (nBM) IN ALZHEIMER'S DISEASE (AD): B.W. Jacobs, T. Ducey, and A.B. Schelbel, Dept. of Anatomy and Psychiatry and the Brain Research Institute, UCLA, CA 90024.

The histopathology of the rostral cholinergic nBM (nBM, diagonal band of Broca, Med. Sect. GE) in AD has been examined. These findings are consistent with reduced NBT, amygdala, and hippocampus. Brain tissue from AD and aged controls were processed for Nissl, thioflavin-S, and acetylcholinesterase (AChE); and immunohistochemically labeled for paired helical filaments (PHF), MAP-tau, amyloid P-component (AP-C), choline-acetyl transferase (ChAT), and glial fibrillary acid protein (GFAP).

A significant proportion of the remaining AChE and Chat positive neurons contained globular NFT. These were demonstrated by thioflavin-S, PHF, MAP-tau, and AP-C. The cores and neurites of senile plaques were positive for PHF. GFAP demonstrated few astrocytes associated with plaque formation. The cholinergic nuclei displayed neurons in various stages of degeneration, many hypertrophied astrocytes, a lack of B-4-amyloid, and a few small clusters of neurons in end-stage gliosis without the presence of neuronal elements. A significant portion of the remaining AChE and Chat positive neurons contained globular NFT. These were demonstrated by thioflavin-S, PHF, MAP-tau, and AP-C but not by AP-C which identified only a few lightly labeled NFT. We suggest the existence of two types of NFT. Those found in the cholinergic nuclei are largely AP-C negative and seem to disappear with neuronal loss; and those from other brain regions which are AP-C positive and tend to accumulate. The finding of styalic, non-accumulating NFT and a paucity of senile plaques and B-amyloid deposition may point to a pathogenic expression unique to the rostral cholinergic nuclei.

389.11

Neurochemical assessments were performed on biopsy samples taken from the right frontal lobe of patients diagnosed with Alzheimer's disease (AD). Prior to the initiation of a venous catheter and pump assembly for the infusion of benzodiazepine as an experimental therapy. Samples were obtained from 6 different medical centers. The pathologists confirmed the AD cases (n = 26) as compared to a group of samples from normal age-matched autopsied controls (n = 21) and age-matched autopsied AD brains (n = 11). All samples were assayed for choline acetyltransferase (ChAT), acetylcholinesterase (AChE), binding to [3H]-quinuclidinyl benzilate (QNB) as an index of total muscarinic cholinergic binding and [3H]-pirepinephrine (PE) binding as an index of M1 receptor subtype binding. Half levels of ChAT activity were decreased to 36% of aged-matched controls, and the loss of activity correlated significantly with the mini-mental state examination, an index of global cognitive function. ChAT activity in AD brain cortex was further decreased compared to controls, indicating a continuous further decline through the course of the disease. AChE followed a similar, less dramatic decline. No differences were seen among the groups in the PE binding was significantly decreased in autopsied AD cases compared to biopsy cases or autopsied controls.


389.13

POSSIBLE NEURONAL PARTICIPATION REVEALED BY PFK(B2) IMMUNOREACTIVITY IN ALZHEIMER DISEASE (AD) PLAQUES.

Dept. of Neurosciences, U.C.S.D., La Jolla, CA 92039.

A previous report suggested involvement of PFK in AD plaques, as we have been studying the spatial relationships between the anti-PFK(B2) labeled component and various cellular populations; i.e., neurons, microglia and astrocytes in this lesion. AD preparations were 40u vibratome sections from the midfrontal cortex and hippocampus. The sections were double immunolabeled with a) anti-PFK(B2)/SMI-31 (neuronal marker), b) anti-PFK(B2)/anti-synaptophysin (presynaptic terminal marker), c) anti-PFK(B2)/B3A1 (microglial marker), and d) anti-PFK(B2)/anti-GFAP (astrocytic marker). Additional sections were double-labeled with anti-amyloid B/AA. The sections were then incubated with a combination of secondary antibodies tagged with FITC and Texas Red for study with the Bio-Rad MRC 600 laser confocal system. Observations of serial optical sections suggest that the PFK(B2) immunoreactive components of the plaques are actually SMI-31 positive, short, sprout-like processes extending from neurons in the area of the diffuse plaque. The cellular processes of astrocytes and microglia were associated with amyloid and neuronal perikarya in the classical plaque. The PFK(B2) immunostained processes in the classical plaques seemed to be closely opposed to amyloid fibrils.

THURSDAY AM

SYMPOSIUM. GENETICALLY MODIFIED CELLS: DEVELOPMENT AND APPLICATION FOR THE NEUROSCIENCES.

B.H. Wainer, The Univ. of Chicago (Chairperson); J.R. Sanes, Washington Univ., St. Louis; D.J. Anderson*, Cal. Tech.; M.R. Capocchi*, Univ. of Utah; F.H. Gage, Univ Calif., San Diego.

We are participating in this symposium to present recent approaches employing genetically modified cells to study areas of specific interest to the neurosciences: cell lineage and development, trophic interactions, and intercellular signaling. Dr. Sanes will describe the application of retrovirus-mediated gene transfer into neural cells in vivo to trace neuronal cell lineage in the embryonic mouse and chick nervous systems. Dr. Anderson will describe the isolation of sympathetic preganglionic neurons, and the demonstration of sympathetic neural processes that generate the major catecholamine-containing derivatives of the neural crest. Dr. Capocchi will discuss the use of gene targeting for the introduction of specific mutations in cells in vitro which can then be employed to generate transgenic animals for evaluation of the consequences of such mutations in vivo. Dr. Wainer will discuss the use of somatic cell fusion approaches to generate cholinergic cell lines from postmitotic embryonic and young adult neurons to study trophic interactions as well as other neuronal processes. Dr. Gage will describe the generation of non-neuronal cell lines transfected with cDNA for either trophic factors or enzymes relevant to specific neurotransmitter systems, the synthesis and secretion of active transgene products by these cells, and the grafting of such cell lines into adult brains to induce long-term functional effects.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION II

392

SYMPOSIUM. DIFFERENTIAL PROCESSING OF VISCERAL AND SOMATIC INPUT IN THE CENTRAL NERVOUS SYSTEM.

R.D. Foreman, Univ. of Okla., HSC (Chairperson); J.M. deGroat, Univ. of Pittsburgh; F. Cervero, Univ. of Bristol; G.F. Gebhart, Univ. of Iowa; R.B. Blair, Univ. of Okla. HSC; M.G. Willis, Univ. of Texas at Galveston.

The objective is to compare and contrast the similarities and differences of visceral and somatic processing in the peripheral and central nervous systems. Dr. deGroat will discuss the similarities and differences in the neurochemistry and anatomy of different pathways to the lumbosacral spinal cord. He will also discuss morphological plasticity in visceral afferent pathways. Dr. Cervero will present studies showing spinal and supraspinal responses of cells delineation of the normal and inflamed colon. Dr. Foreman will compare and contrast the organization of spinal afferent cells that receive cutaneous, muscle and visceral input and project to the oral and caudal regions of the ventral posterior lateral thalamic nucleus. Dr. Blair will summarize how different regions of the medullary reticular formation integrate visceral, primarily cardiac, and somatic information. Dr. Willis will discuss how thalamic neuronal structures integrate visceral and somatic input. We will conclude with his perspective concerning future directions that need to be pursued to provide the most effective development of this field.

393.1

PRO-OPTOMELANOCORTIN (POMC) NEURONS IN THE RAT MEDIOBASAL HYPOTHALAMUS (MBH) PROJECTING TO THE MEDIAL PREGENITAL AREA (MPG) ARE SYNAPTIC TARGETS OF GAABAERIC TERMINALS.

F. Naito*, M. Shangbrough* and C. Lenhossék.
Dept. of Obstetrics and Gynecology and Section of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT. 06510.

We have previously shown that PVN-pedotopically-containing neurons project a direct link between the arcuate nucleus (AN) and MPG neurons. The AN contains GABAergic interneurons which could control the AN POMC neurons. To test this hypothesis, intrasegmental injection of retrograde tracer, horseradish peroxidase (HRP) and a cholinergic neuronal marker, sensory neuron-specific enolase (SNSE) were injected into AN. Neurons retrogradely labeled with HRP were predominantly within the arcuate nucleus and extended into the ME. The SNSE-labeled neurons were located within the arcuate nucleus and extended into the ME. The SNSE-labeled neurons were located as far as the habenula. These results indicate that the AN-POMC neurons may be a target of the ME-AN pathway. These findings would suggest that the ME-AN pathway may be involved in the regulation of pituitary ACTH release.

393.2

EVIDENCE THAT NEUROPEPTIDES Y (NPY) PLAYS A ROLE IN THE PUBERTAL INCREASE IN LHRRH RELEASE IN THE FEMALE MONKEY.

A.C. Gage & L. Toran-Allerand.

An increase in pulsatile LHRRH release from the hypothalamic is critical for the initiation of puberty. However, the neural mechanism controlling this pubertal increase in LHRRH is unknown. Since we found that NPY plays a role in the control of pulsatile LHRRH release in adult monkeys, we examined whether NPY contributes to the increase in LHRRH release that occurs during puberty by using push-pull perfusion of the stalk median eminence (S-ME). In the first experiment, endogenous NPY release in the S-ME of 10 prepubertal (12-20 mo) and 11 midpubertal (21-40 mo) monkeys was measured by RIA. No developmental changes in NPY release were observed; mean NPY release was 204.4±24 and 234.8±80 pg/ml for pre- and midpubertal monkeys, respectively. Thus, in the 2nd experiment we tested whether the responsiveness of the LHRRH neuronal system to endogenous NPY changes during puberty. NPY (10 & 100mM) or vehicle was infused to the ME for 10 min through the push canula and LHRRH levels in samples collected at 10 min intervals were measured by RIA. In prepubertal monkeys (n=5), mean LHRRH release was 1.3±0.2 pg/ml and was not affected by infusion of either NPY or vehicle. In contrast, in midpubertal monkeys (n=5) NPY infusion significantly stimulated LHRRH release (p<0.001) from 4.3±1.4 to 6.5±1.9 pg/ml after 10mM NPY and from 3.6±1.1 to 6.1±1.4 pg/ml after 100mM NPY. Vehicle infusion did not affect LHRRH release. These results suggest that while NPY levels do not change, an increase in the responsiveness of the LHRRH neuronal system to NPY occurs during the pubertal process. Therefore, we conclude that the pubertal increase in pulsatile LHRRH release is, in part, due to an increased sensitivity to NPY. (HD11355, RR00167 & GM07507).
NPTY may enhance the secretory response of gonadotropin to LH during the hypophysiotrophic function of the LH surge. To test this hypothesis, effects of NPTY on LH-stimulated LH secretion were assessed in perfused hypothalamic (PB)-anesthetized, proestrus rats. Female rats were fitted with arterial catheters on diestrous. On proestrus, hourly blood samples were collected from 0900-2100. At 1330h, rats received PB (40 μg/kg, iv) and then received continuous PB-LH release, or saline. Every 30 min from 1400-1800, PB-treated rats received i.v. pulses of LH (15, 150, or 1500 ng/pulse) or saline. In each of these experiments, mean level of LH was measured. Plasma samples were analyzed by LH RIA. In PB-treated rats receiving vehicle pulses only, LH surges were completely blocked. Pulsatile LH treatments at 15, 150 and 1500 ng/pulse produced sub-physiological, physiological, and supra-physiological LH surges, respectively. These results support the hypothesis that NPTY operates as a neuroendocrine modulator during generation of the proestrual LH surge. (Unpubl.)

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION II


392.6 ELEVATION OF PROOPOMELANOCORTIN mRNA LEVELS IN THE RAT Ovary by GONADOTROPIN STIMULATION. A.H. Kaynad and M.H. Molony. Divisions of Neurosciences and Reproductive Biology, Oregon Regional Primate Research Center, Beaverton, OR 97006.


392.8 THE SEXUALLY DIMORPHIC DISTRIBUTION OF SUBSTANCE P IN SPECIFIC ANTERIOR PITUITARY CELL TYPES. Elaine R. Brown, Kevin A. Bank*, and James E. Krause. Dept. of Anatomy & Neurobiology and Pathology, Washington University School of Medicine, St. Louis, MO 63110.

Substance P (SP) immunoreactivity has been detected by radioimmunoassay in the rat anterior pituitary (AP) as a prepropeptide. The present report provides evidence that there is a correlation between the levels of SP in the pituitary and the levels of pituitary hormone secretion.

392.9 SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990 THURSDAY AM


We have previously shown that vasopressin (VP) and opioid (OP) neurons synapse directly on gonadotropin-releasing hormone (GnRH) neurons in juvenile monkeys. To investigate relationships between VP and OP-immunoreactive (-IR) neurons, we performed double label immunostaining on vibratome sections through the SON and PVN of cynomolgus monkeys. VP and POMC-IR neurons were prelabeled (Sci. Neurosci., 14:439, 1988). In the SON, beaded OP-IR fibers innervated about 33% of the VP-IR cell bodies anteriorly and 10% posteriorly. In the PVN, clusters of OP-IR terminals were located around major processes arising from about 42% of the VP-IR neurons. EM confirmed that OP-IR terminals made mostly symmetrical synapses on both NEU-IR and NEU-IR dendrites.

These results provide anatomical evidence of direct OP modulation of NEU VP release from the median eminence and posterior pituitary, and of non-NEU, intrahypothalamic VP neuronal activity. We have now identified OP-IR innervation of VP, oxytocin, dopamine and GnRH neurons in the monkey hypothalamus. It is not known whether these OP-IR afferents arise from the same POMC neurons, whether they release the same POMC product at any synapse, or whether the peptides impart stimulatory or inhibitory post-synaptic effects. Other data have suggested CRF may coexist in some VP-IR neurons, and that CRF acts via VP neurons to stimulate OP neuronal activity and to inhibit GNRH release. Taken together, these results suggest that stressful stimuli initiate a complex combination of peptideergic neurointeractions which regulate endocrine stress to stress in primate.


393.8 THE SEXUALLY DIMORPHIC DISTRIBUTION OF SUBSTANCE P IN SPECIFIC ANTERIOR PITUITARY CELL TYPES. Elaine R. Brown, Kevin A. Bank*, and James E. Krause. Dept. of Anatomy & Neurobiology and Pathology, Washington University School of Medicine, St. Louis, MO 63110.

Substance P (SP) immunoreactivity has been detected by radioimmunoassay in the rat anterior pituitary (AP). The present report provides evidence that there is a correlation between the levels of SP in the pituitary and the levels of pituitary hormone secretion.

393.9 SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990 THURSDAY AM
392.9


The critical period for sexual differentiation of the brain in the developing lamb lasts several hours before birth. Exposure to steroids during mid-gestation alters the control of GnRH secretion after birth (Wood and Foster, unpublished). We have examined GnRH neurons in male and female lambs during the critical period to determine if sexual dimorphism of these neurons exists. The number and distribution of GnRH-containing neurons from mid-gestation (85 days) male and female lambs were compared (n=6 each). Immunoreactive cells were labelled using LR-1 antiserum (R. Benoit) with the Vectastain ABC kit. GnRH neurons were localized in 60 um coronal sections in a block of tissue extending from the diagonal band of Broca to the rostral mamillary bodies. Neither the pattern of distribution nor the estimated number of GnRH neurons in male and female fetuses differed (185±25 vs 177±35, P>0.05), and the total number of cells was similar to that reported for the adult ewe (Lehman, M. et al, J Comp Neurol, 244:19, 1986). Unlike the adult, substantial numbers of GnRH neurons in the fetus were present caudal to the optic chiasm (males 28% of total, females 37%, P>0.05). These data indicate that the distribution of GnRH neurons may change during later stages of development and is not sexually differentiated during the critical period. (Supported by USDA 89-37240-45661)

393.11

INCREASED SUBSTANCE P AND RECEPTOR B GENE EXPRESSION IN HYPOTHALAMIC POMONA PAL POSTANOUS JUNE II, RANCE, and W.S. Young III. Department of Pathology, University of Arizona College of Medicine, Tucson, AZ 85724, and Laboratory of Cell Biology, NIH, Bethesda, MD 20892.

We have recently shown a striking hypertrophy of neurons containing estrogen receptor. Neurokinin B (NEK) and Substance P (SP) mRNAs in the infundibular nucleus of postpubertal (POSTM) women (Rance et al., 1990, J Clin Endo Metab. In press; Rance and Young, 1990, Endo Soc Abst). In the present study we examined if POSTM neuronal hypertrophy is accompanied by increased gene expression. Sections from hypothalami of premenopausal (PREM; n=10) and POSTM (n=6) women were incubated with [3H]-labeled synthetic oligonucleotide probes complementary to NEK and SP mRNAs. The mean cross-sectional areas of POSTM infundibular neurons labeled with the SP and NEK probes increased to 180±k and 196±k of PREM values, respectively. Autoradiographic grain densities (ngains/100pm2) for both SP (1.8±0.8) and NEK (130±4) were also elevated in the POSTM women. The most striking findings were 4-fold (SP) and 15-fold (NEK) increases in the numbers of labeled neuron/case area in the POSTM infundibular nucleus. These data suggest the hypothesis that POSTM neuronal hypertrophy is due to removal of the inhibitory feedback of ovarian steroids and demonstrates that human menopause is associated with marked increases in hypothalamic neuropeptide gene expression.

BASAL GANGLIA AND THALAMUS VI

394.1


Evidence from several experiments necessitates a revision of the widely believed concept that dopaminergic supersensitivity can be explained primarily by increases in striatal D2 density. The dopamine D2 receptor, given a minimal 6-OHDA lesion of the dopamine (DA) pathways and subsequent chronic treatment with the selective D2 agonist eticlopride show vigorous rotation contrateral to the lesion despite the fact that D2 density is symmetrically equal in both striata. Chronic treatment of neurologically intact rats with haloperidol, which increases striatal D2 density to the same degree (25-40%) as a 6-OHDA lesion, results in a modest (2-fold) increase in sensitivity to the behavioral effects of the D2 agonist agonist apomorphine or the mixed D1/D2 agonist amphetamine, in marked contrast to the 20-40 fold increase in sensitivity observed following 6-OHDA lesions. (Supported by NIH HD07133)

Alternatively, the profound supersensitivity of 6-OHDA- or eticlopride-treated rats to DA agonists may relate to the breakdown of the normal D1/D2 synergism. First, treatment with 5 daily injections of eticlopride (1 mg/kg s.c.) induces breakdowns in the synergistic effects of D1 and D2 agonists but does not alter striatal D1 or D2 receptor density. Second, intact rats given a fixed, high dose (20 mg/kg i.p.) of the D1 agonist SKF 38930 show a dose relationship for stereotypic behavior qualitatively similar to that observed in eticlopride-treated rats not given the D1 agonist. Thus, two types of DA receptor supersensitivity may be discerned: a modest one associated with D2 receptor supersensitivity and a profound one that is not dependent on increased D2 or D1 receptor density and, instead, is associated with a breakdown of D1/D2 synergy. We hypothesize that this profound type of dopaminergic supersensitivity results from the liberation of this receptor subtype from control by the D1 receptor. This reformation may have important implications for understanding certain aspects of tective dyskinesia, Parkinson's disease, and schizophrenia.

394.2


We have shown by immunohistochemistry and in situ hybridization that the psychomotor stimulant drugs, cocaine and amphetamine, induce the expression of the immediate early gene (IEG) c-fos and c-jun in the striatum of rats treated systemically with these drugs. We now report that c-Jun expression is also induced in the striatum of rats treated systemically with cocaine (25 mg/kg) and amphetamine (5 mg/kg), and extended 1 hr later. We compared the patterns of mRNA induction in the striatum for these IEG in serial sections.

The in situ hybridization autoradiograms show strong induction of c-Jun by both cocaine and amphetamine, and demonstrate that this induction was paralleled by activation of c-jun. By contrast, there was no evidence of mRNA induction of c-fos by either cocaine or amphetamine in the striatum of the same brains. These findings establish that, among IEGs of the leucine zipper family, at least two, Jun B and c-jun are coordinate induced within the striatum by psychomotor stimulation. These two IEGs may reflect the broadened effects of such drugs. Cortical lesions also coinduced Jun B and c-jun in the ipsilateral cortex, suggesting a more general occurrence of this form of gene coexpression. Supported by The Sower Institute, NIH RO1 DA051792, NARSAD, United Parkinson Foundation, and MRC of...
394.3 STRESS-INDUCED ACTIVATION OF THE IMMEDIATE-EARLY GENES c-FOS AND c-JUN IN STRIATUM BY D1- AND D2-SELECTIVE DOPAMINE AGONISTS


The striatum is a key component of the basal ganglia and is the primary site of dopaminergic input from the substantia nigra and ventral tegmental area. The striatum is divided into three main compartments: the dorsolateral striatum, the ventromedial striatum, and the ventral pallidum. These compartments have distinct functions and receive inputs from different regions of the cerebral cortex. The activation of the immediate-early genes c-FOS and c-JUN in the striatum during stress has been well documented. These genes are upregulated following various stressors, and their activation is thought to be involved in the neurobiological changes that occur following stress.

394.4 REGULATION BY D1 AND D2 DOPAMINE RECEPTORS OF STRIATONIGRAL AND STRIATOPALLIDAL PEPTIDE mRNA LEVELS

C.R. Grefer, K.P. Engebretson, I. Tse, L. C. Mah, P. J. Witte, and D. McVittie, Lab. of Cell Biology, National Institute of Mental Health and Experimental Therapeutics Branch, NINDS, National Institutes of Health, Bethesda, MD 20892.

D1 and D2 dopamine receptor agonists have been shown to regulate the expression of several genes in the striatum, including the immediate-early genes c-FOS and c-JUN. These receptors are known to modulate the expression of genes involved in various cellular processes, including protein synthesis, cellular proliferation, and survival. The regulation of gene expression by dopamine receptors is thought to be involved in the pathophysiology of various neurological disorders, including schizophrenia and Parkinson's disease.

394.5 EXPRESSION OF GFAP AND J1/TENASCIN IN NORMAL DEVELOPING NEOSTRIATUM AND AFTER 6-OHDA LESION

T.F.OBrien, K. Harrington, A. Faisser, M. Schachner, and D.A. Bean, Dept. of Anat. and Neurosci., Univ. of Tenn., Memphis, and Dept. of Neurobiol., Univ. of Heidelberg.

GFAP (glial fibrillary acidic protein) and J1/tensin are important markers for the developing and adult striatum. GFAP is expressed in astrocytes, which are the main supporting cells in the brain, while J1/tensin is expressed in neurons. The expression of these proteins is altered in various neurological disorders, including Huntington's disease and Parkinson's disease. The expression of GFAP and J1/tensin in the developing striatum is important for the proper development of the striatum and its associated functions.

394.6 A COMPARISON BETWEEN DORSOLATERAL AND VENTROMEDIAL STRIATAL PATHWAYS THROUGH THE MONKEY BASAL GANGLIA

S.N. Haber, E.L. Lynch, and J.Mitchell, Department of Neurobiology and Anatomy, University of Rochester, Rochester, N.Y., *VA Medical Center, Syracuse, N.Y.

The striatum is a complex structure that is divided into two main regions: the dorsolateral striatum and the ventromedial striatum. These regions have different functions, and their pathways are involved in various neurological disorders. The comparison between these two regions is important for understanding the pathophysiology of these disorders.

394.7 A DYADIC PATCH-MATRIX SCHEME DOES NOT ADEQUATELY DEFINE THE HISTOCHEMICAL HETEROGENEITY OF THE HUMAN STRIATUM

G.G. Basile, G. Ko, and N. Kowald, Neurol. Therapy Service, Massachusetts General Hospital, Boston MA 02114.

The human striatum is a complex structure that is divided into two main regions: the dorsal striatum and the ventral striatum. The dorsal striatum is involved in motor function, while the ventral striatum is involved in reward and motivation. The histochemical heterogeneity of the human striatum is important for understanding its function and pathology.

394.8 ALL SERIALLY ANALYZED CHOLINE ACETYLTRANSFERASE IMMUNOREACTIVE NEURONS SHOW DIRECT SOMATIC OR DENDRITIC CONTACTS WITH UNLABLED SPINY NEURONS IN ADULT RAT CAUDATE-PUTAMEN NUCLEUS


Direct somatic and dendritic contacts between neurons mediate electrical coupling and influence the level of expression of choline acetyltransferase (ChAT) in certain areas of the brain. These contacts are important for the proper function of the brain and are thought to be involved in various neurological disorders.
394.9

In hemiglosus after frontal cortical damage there is decreased activity in motoneuronal and basal ganglia as determined by quantitative 2-deoxyglucose autoradiography (2DG) in the monkey. The effect of continuous manual motor behavior on 2DG uptake by these structures was tested in neglect patients exerting cueing, measured, and timed forces with their hands. 2DG uptake by these structures was tested in neglect patients exerting cueing, measured, and timed forces with their hands. 2DG uptake by these structures was tested in neglect patients exerting cueing, measured, and timed forces with their hands.

394.10
THE NORADNERGIC INNERVATION OF THE THALAMIC RETICULAR NUCLEUS AND THE BASAL REGIONAL NUCLEUS IN RATS. C. Asanuma, Laboratory of Neurophysiology, NIH, NIMH Animal Center, Polkerville, MD. 20837.

Pharmacological and coirnoradrenaline injections into the thalamus and basal ganglia produce large increases in electrical activity by certain of the thalamo-cortical pathways. The current study was focused on some of the other inputs to these structures and their relationship to brainstem outputs. This is because these additional inputs may indirectly influence the state dependent gaiting of signal flow through the nuclei of the dorsal thalamus. Neurons in several sites were identified by the response to the 2DG and the electrical activity in the thalamic nucleus, have discharge properties which are closely correlated with changes in arousal levels. In the current study, dopamine-D2-receptor (DB2) immunohistochemistry was used to examine the activation patterns of noradrenergic axons within the TRN and the basal region nucleus. This was followed by intracellular injections of Lucifer yellow and 2DG slices to examine the correlation of noradrenergic axons to neurons within these two areas.

394.11
INTRACELLULAR AUTOSTIMULATION OF IN VITRO GUINEA-PIG THALAMIC NEURONS BY UTILIZING A HARDWARE BIOELECTRIC RECORDING SYSTEM. Y. Yaron1 and R. Lind9, Dept. of Physiol & Biophysics N.Y.U. Med. Cn., N.Y., Dept. of Neurobiology, Hebrew University, Jerusalem, Israel.

A waveform generator simulating the feedback activity from the thalamic nucleus was constructed to explore the role of hyperpolarizing potentials in the generation of oscillatory thalamic activity. IPSP-like waveforms were simulated by controlling the membrane potential and rhythmicity of the neurons. The thalamic sensory nuclei were identified by the recording electrode. The injection was triggered by TH spikes and was proportional to the number of spikes in the burst. The "IPSP" parameters best suited to controlling rhythmicity were then determined. The frequency was programmed to the given value. A brief depolarization of TH neurons could trigger prolonged rhythmic activity at a frequency determined by the duration of the hyperpolarization pulses. This activity was transiently maintained by a burst of spikes. The excitations of the superior colliculus of the thalamus involved by the depolarizations may be required for sustained rhythmicity during the burst. The interburst interval limited the burst duration. Thus, the interval successively shortened, the LTS decreased ending the burst—indeed of its initial frequency. If the LTS of TH cells was held depolarized, inhibitory feedback could induce high frequency oscillations, by modulating the repetitive firing into well ordered trains whose frequency (10 to 50 Hz) depended on the amplitude and duration of the "IPSP". Thus 1) Feed-back hyperpolarizing currents are necessary and sufficient to generate and maintain thalamic neuronal drive. It was further demonstrated that the oscillatory frequency is not affected by the duration of the "IPSP". As example, the neuronal drive of the thalamus can be established by the oscillations in the thalamus.

394.12
INTRACELLULAR RESPONSE OF RAT NUCLEUS ACCUMBENS NEURONS FOLLOWING STIMULATION OF HIPPOCAMPAL INPUTS IN AN IN VITRO SLICE PREPARATION. C.A. Pennartz and S.T. Kital, Department of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, TN 38163.

The aim of the present study was to analyze responses of neurons of the nucleus accumbens to stimulation of the hippocampus, which contains affrents from the hippocampal formation. Using electrodes filled with 0.5 M K-methylsulphate and 2 M bicuculline, recordings were made from 52 neurons (neuronal potential: -68 ± 2 (SD) mV, input resistance 42 ± 4 (MΩ)). After histological processing with avidin-Texas Red, 4S labeled neurons were identified as dopaminergic neurons. A depolarizing and inhibitory postsynaptic potential (IPSP) following stimulation of the fornix was recorded in 48 neurons. Using intracellular injection of positive DC current and Na channel blocker, APV, this IPSP could be dissociated into an EPSP reversing its polarity at -11 to +15 mV and a bicuculline-sensitive IPSP reversing at -70 to +50 mV. The threshold for the IPSP was lower than for the EPSP. Furthermore, the IPSP onset latency was consistently shorter than the spike latency. The data indicate that medium spiny neurons receive excitatory monosynaptic inputs from the subiculum. In addition, fornix stimulation most likely activates a feedforward inhibitory pathway in the nucleus accumbens. (Supported by Grant USPHS NS 233886 and 20782 to S.T. Kital.)

CITRULLINE CATHODAL CHANNELS III

395.1

We have used action potential waveforms (APW) as voltage-clamp command pulses to investigate Ca++ current activation by voltage transients which are more natural than conventional square steps. Traces have been labeled APW (upward) and whole-cell Ca++ currents (downward) recorded in a chick sensory neuron. Currents were recorded before and during exposure to 3 mM amiloride, which selectively blocks T-type current. Ca+++L = 2mM. As illustrated, with a normal, bell-shaped Ca++ current, the current to the total is much larger than would be expected, since when currents are activated with conventional voltage steps T is much smaller than the high-voltage activated current (HVA-M). Two factors can account for this low threshold and slower deactivation kinetics of T. The latter leaves T channels open after prolongation, when the driving force is great. We also find that increasing APW duration causes a much larger increase in HVA than in T current. Supported by NS08373 and NS26416.

395.2

A voltage-dependent Ca current can be elicited in cerebellar granule cells mechanically dissociated at PNS and maintained 5 days in culture (DEMEM + 25 mM KCl). With 10 mM BaCl2 in the external medium, a current was elicited above -20 nV and reached a mean peak value of 150 pA at +10 nV. The total current (up to 90%) was half inactivated by holding the membrane potential at -70 mV. It was further demonstrated that the current was highly regenerative and could be associated with a non-linear activation curve. The current was also sensitive to the dihydropyridines (DHP) as recorded in cell attached condition (10 μM BaCl2). Two channel types were identified, each displaying such a sensitivity. A = 25 pS L-channel type recorded: its low opening probability in control conditions (<0.001) was greatly increased by depolarization and addition of 10 μM Bay 6, 644, in addition, a spontaneous 5-7 pS was observed at potentials above -50 mV which open time was greatly enhanced by both depolarization and addition of 10 μM Bay 6. 644. The latter channel might account for the DHP-sensitive Ca entry commonly measured in flux experiments.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

In order to learn about the biophysical and pharmacological properties of Ca$^{2+}$ channel currents present in CGN, we measured transmembrane currents in whom cell configuration using the patch clamp technique. Currents elicited by a holding potential of -90 mV in the presence of BaCl$_2$, 20 mM. Cadmium (100 µM) blocked completely the Ca$^{2+}$ current, while neither amiloride (500 µM) nor w-conotoxin (10 µM) affected the current. While nifedipine (300 nM) partially blocked the current, the current from the funnel-web spider venom (FTX) (Llinas et al., PNAS, 1989, 89:1689) blocked most of the current.

Single-channel experiments, performed in cell-attached configuration with 110 mM BaCl$_2$, revealed the presence of a main conductance level of about 20 pS (whose frequency was increased when BAY K 8644 I µM was added to the bath solution) and in some cases other lower conductance levels. Biophysical and pharmacological properties suggest that in cultured CGN at least two Ca$^{2+}$ channel species can be characterized. One can be characterized biophysically as L-type (Nowicki et al., Nature, 1985, 316:440) and the other with a lower conductance is similar to the P channel described by Llinas et al. (PNAS, 1989, 89:1689).

395.4 VOLTAGE-DEPENDENT AND PHARMACOLOGICAL PROPERTIES OF CALCIUM CURRENTS IN ISOLATED RAT CEREBELLAR PURKINJE CELLS. P.E. Hockberger and L. Youssf. Dept. of Physiology, Northwestern Univ. Medical School, Chicago, IL 60611.

Whole-cell calcium currents were recorded from identified Purkinje cells isolated from 1-2-week-old rats, as described previously (Hockberger et al., Soc. Neurosci., Abstr. 15: 1149, 1989). Both high-threshold (HT) and low-threshold currents were evaluated. Although HT and LT currents did not appear during the second postnatal week. This report focuses on the properties of the HT current. The current amplitude was dependent upon the holding potential, e.g., peak amplitude was 50-75% larger when held at -80mV compared with -40mV. Regardless of the holding potential, the threshold for activation was ~20mV (±5mV) and the peak amplitude was evoked around 0mV (±5mV). During voltage steps the degree of current inactivation was ~10% during the initial 50ms, but it reached 20-40% after 400ms. Recovery from inactivation was slow (T = 1-2 min). Local application of calcium channel antagonists via micropipet revealed that nifedipine (NIF), w-conotoxin-GVIA (CTX), or a synthetic analog of funnel-web spider toxin (FTX) reduced the HT current in a dose-dependent and reversible manner. Concentrations of 80-100µM were obtained when applying NIF, CTX, or FTX at 10-5M and stepping from either -40 or -80mV. Including 8-bromo-cGMP (10-5M) in the internal solution did not affect any of the measured parameters.

This research was supported by NIH grants NS-26915 and NS-17489. The FTX was kindly provided by Pfizer Central Research (Groton, CT) and Natural Product Sciences (Salt Lake City, Utah).

395.5 ISOLATION OF A CALCIUM CHANNEL FROM MAMMALIAN CEREBRAL TISSUE USING SYNTHETIC FTX. B. D. Cherksey, M. Sugimoto and P. Linds. Dept. of Molecular and Cell Biology, U.C. Berkeley, Berkeley, CA 94720.

The specific blocker of the P-type channel was isolated from the venom of Apohelengos aperta (a neurotoxic venom). The venom was used to isolate a neuronal membrane protein which when reconstituted into a lipid bilayer gave channel activity. Small amounts of purified FTX gave further more biochemical studies unsatisfactory. Based on our structural analysis of FTX, we have synthesized a polyamine ("synthetic FTX") (Cherksey, et al., BioL Bull, 1989) with specific P-channel blocking characteristics. The availability of P-channel FTX has made possible further studies of the P-channel protein. Synthetic FTX was coupled to Sepharose. Membranes from cow cerebellum homogenate, solubilized in 3% sodium cholate, were subjected, batch-wise, to affinity chromatography on Synthetic FTX gel. Purified protein was eluted with 1M calcium chloride. The volume was reduced. Portions of the material were taken and reconstituted into liposomes. Functional activity was determined using the lipid bilayer technique. The purified protein had channel-like activity, blocked by FTX, indistinguishable from that obtained using purified FTX as the gel ligand. SDS polyacrylamide gel electrophoresis of the purified protein revealed a single sharp band in the 120,000 Da region. Similar results were obtained using size-exclusion HPLC. Under reducing conditions only 2 bands were seen using SDS-PAGE; one band at 70,000 Da and a broad band over the 200,000-300,000 Da region. On HPLC, the broad band was resolved into 2 bands: 27,000 and 24,000 which could represent either 2 distinct proteins or 1 protein with differing states of glycosylation. These results differ substantially from those reported for the other known calcium channels. The purified protein was injected into rabbits and immune serum obtained. The antibody was found to react specifically with the P-channel protein. Both the functional activity and structural characteristics of the isolated P-channel protein differ from those reported for the L and N type channels and strengthen the identification of the P-type channel as a distinct molecular entity.

395.6 NEW w-AGATOXIN CALCIUM CHANNEL ANTAGONISTS FROM FUNNEL WEB SPIDER VENOM ACTIVE IN THE AVIAN AND MAMMALIAN BRAIN. J. L. Vennema* and M. Adams. Dept. of Entomology, Univ. of California, Riverside, CA 92521.

Venom of the funnel web spider A格力ngos a格力pta is a rich source of voltage-sensitive calcium channel (VSCC) antagonists known as w-agatoxins. First recognized for their inhibition of presynaptic VSCC at insect neuromuscular junctions, the w-agatoxins also are potent and selective antagonists of VSCC in vertebrate excitable tissue (see also Linds et al., M Nile, et al., this meeting). Reversed-phase liquid chromatography (RPLC) of crude A. a格力pta venom yields a broad w-agatoxin elution profile characterized by inhibition of specific w-agatoxin GVIA (w-CgTX) binding to chick synapsosomal membranes. Using this assay as an assay for purification, we isolated w-Ag-AIIa, an 8.5 kDa polypeptide constituting about 1% of total protein in the venom. w-Ag-AIIa is a dose-dependent inhibitor of w-CgTX binding (EC50 = 10-20 nM) to a maximum of 100% above 60 nM. w-Ag-AIIa also inhibits potassium-induced synaptic Ca$^{2+}$ flux with an EC50 of 1.5 µM. However, saturating concentrations of w-Ag-AIIa block only 67% of total flux, while w-CgTX blocks 100% of the flux response. Therefore, 25-30% of usual w-CgTX flux in chick synapsosomes appears to be insensitive to w-Ag-AIIa.

Potassium-induced Ca$^{2+}$ flux in rat brain synaptosomes is relatively insensitive to w-Ag-AIIa and w-CgTX antagonism. However, new w-agatoxin currently under study suppress rat synaptic Ca$^{2+}$ flux at apparent nanomolar concentrations. Further analysis of the specifics and modes of action of the w-agatoxin promises to yield useful information on the subclassification as well as tissue and phylogenetic diversity of VSCC.

Supported by NIH grant NS34472.


The peptide w-Aga-IIIA from the venom of Agelenopsis a格力pta was tested on various calcium conductances in freshly dissociated neonatal rat DRG neurons, using whole cell recordings of Ba$^{2+}$ currents. The toxin showed high-threshold current with high potency (Kd = 1.2 µM) but a low fraction of the peak current was blocked (12%) (Fig B). However, in 100 mM (w-Aga-IIIA blocks >98% of the L-type Ca current. Unlike therapeutically used Ca antagonists, block by w-Aga-IIIA is voltage-independent; channel kinetics are not changed and channel availability is not shifted. Block of L-type Ca channel by w-Aga-IIIA is partially reversible.


The peptide w-Aga-IIB from the venom of Agelenopsis a格力pta was tested on various calcium conductances in freshly dissociated neonatal rat DRG neurons, using whole cell recordings of Ba$^{2+}$ currents. The toxin showed high-threshold current with high potency (Kd = 1.2 µM) but a low fraction of the peak current was blocked (12%) (Fig B). However, in 100 mM (w-Aga-IIB blocks >98% of the L-type Ca current. Unlike therapeutically used Ca antagonists, block by w-Aga-IIB is voltage-independent; channel kinetics are not changed and channel availability is not shifted. Block of L-type Ca channel by w-Aga-IIB is partially reversible.

- **A**: 0, 1, 4, 16 mM w-Aga-IIIA
- **B**: 280 nM w-Aga-IIB

- **L-type DRG neurons**
  - **Kd = 1.2 µM**
  - **Resistant current**

- **w-Aga-IIIA** blocked wth similar potency to the N-type current of frog sympathetic neurons (n=20), the L-type current of rat ventricular cardiac cells (n=4), and high-threshold Ba$^{2+}$ currents in neonatal rat hippocampal neurons (n=3). In contrast, T-type currents in rat DRG neurons were not affected (200 mM w-Aga-IIIA, n=3).
395.9 Funnel-web spider toxin (FTX) selectively blocks the sustained, but not the transient, calcium current in retinal horizontal cells. J.M. Sullivan* and E.M.L. Lastar, Deps. of Physiology and Ophthalmology, Univ. of Utah School of Medicine, Salt Lake City, Utah, 84138.

Using the whole-cell patch-clamp technique, we have identified two separate calcium currents—one sustained (I(Ca,S)) and one transient (I(Ca,T))—in rod outer segments (ROS) isolated from adult white rats. Calcium current was enhanced using 10 mM extracellular Ca, while Na+ and K+ currents were pharmacologically suppressed. The large transient Ca current is similar, but not identical, to the T-current described in other preparations: I(Ca,T) activates and inactivates at -60 mV, is inactivated at a holding potential of -40 mV and is carried less well by Ba2+ than Ca2+. Unlike the T-current, I(Ca,S) is not preferentially blocked by Ni2+.

These data suggest that the T-current has been cloned in HCs, or in a non-mammalian vertebrate preparation. The sustained Ca current is similar, but not identical, to the L-current described in many preparations: I(Ca,S) activates at -50 mV, is larger when Ba2+ replaces Ca2+ and is enhanced by the dihydropyridine agonist BAY K 8644 (calcineurin). Unlike the L-current, I(Ca,S) is not preferentially blocked by Ca2+ and, nor is it reduced by W-conotoxin fraction GVA (a gift from Dr. B. M. Oliveras). FTX, a factor isolated from the venom of the funnel-web spider (Linaus et al., P.N.A.S. 86:1689, 1989), selectively blocks I(Ca,S) at very low concentrations: a 1:3000 dilution of the column fraction containing FTX blocked 60% of the sustained current, while a 1:10,000,000 dilution blocked 10% of this current. This block was maintained even after washing with FTX-free Ringer for up to 20 min. These results strongly suggest that sustained Ca currents are carried through two different and unique types of channels, and support the notion that there is a wide variety of Ca channels among different species and tissue types.

This work was supported by N.I.H. Grant EY05972 to E.M.L.

395.11 Biochemical Characterization of a High Affinity [3H]Ryanodine Receptor From Rabbit Brain Membranes. P.S. McPherson and K.P. Campbell. Howard Hughes Medical Institute and Program in Neuroscience and Dept. of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242.

The skeletal muscle receptor for the plant alkaloid ryanodine has been shown to be identical to the Ca2+ release channel of the sarcoplasmic reticulum. High affinity [3H]ryanodine binding has been recently demonstrated in isolated brain membranes (Ashley, R.H., J. Membr. Biol. 117:193, 1989). We have shown that [3H]ryanodine binding is enriched in membranes from the hippocampus but is significantly lower in membranes from the brain stem and spinal cord. Approximately 50% of [3H]ryanodine labeled receptor is solubilized from brain membranes using CHAPS phosphatidylycholine containing 1 mM NaCl. The brain [3H]ryanodine receptor comigrates through sucrose gradients with the skeletal receptor as a large complex ("QSR"). Solubilized receptor is specific for high affinity, as judged by gel filtration and by competitive inhibition by heparin-agarose chromatography followed by sucrose density gradient centrifugation. A protein of ~40,000 Da is enriched in peak [3H]ryanodine binding fractions of heparin-agarose eluates and sucrose gradients, and is cross-reactive with antibodies raised against the skeletal muscle ryanodine receptor. We propose that the ~40,000 Da protein is the brain form of the high affinity ryanodine receptor and that it functions as a Ca2+ release channel in brain endoplasmic reticulum. Kevin P. Campbell is an Investigator of the Howard Hughes Medical Institute.


The major protein component of a dihydropyridine-sensitive calcium channel from skeletal muscle was cloned and sequenced (Tanabe, T., et al., Nature 328, 313-318). The primary structure suggests the occurrence of four internal repeats, each containing six presumably α-helical transmembrane segments. We designed a protein that mimics a pore-forming structure of the calcium channel; a carboxy terminal domain (KCGRI) (Muster, J. et al., 1988, Zentrakonf.44, 711-715) was used to direct the assembly of a bundle comprised of four identical 22-mer peptides with sequences corresponding to transmembrane segment IV3 (DPYPWQPPLFVGILSTV). This synthetic protein forms calcium-selective channels in lipidd bilayers. At pH 7.2, the single channel conductance in symmetric 50 mM CaCl2 and 50 mM BfCl2 is 1.3 ± 0.2 nA and is decreased to 1.5 ± 0.1 nA in 100 mM MgCl2. Channels are blocked by 100 mM 4,4′-dimethyl-2,2′-pyridine, 100 mM verapamil and 100 mM of the local anesthetic analogue of lidocaine. A different single channel protein containing four peptides with sequences corresponding to segment IV1 (DPYP) is larger and more abundant than FGRI, but is not functional in lipidd bilayers.

Supported by NSGMS (GM-42340), NIMH (MH-44687 and MH-07778) and ONR (N0014-89-2-1130).
CONANTOKIN: STRUCTURE, FUNCTION AND DEVELOPMENTAL SPECIFICITY. L. J. Cruz F., C. Abadie, J. M. Mcintosh, J. F. Hernandez* and J. Rivier*. Marine Science Inst., Univ. Philippines, Diliman, G. C. 1101; Dept. of Biology, Univ. Utah, Salt Lake City, UT 84112; Sakai Institute, La Jolla, CA 92037.

Fish-hunting marine snails produce numerous biologically active peptides; of particular interest are conantokins which inhibit the glutamate receptor of the NM receptor. First detected as components which induce sleep in young mice but cause hyperactivity in older mice, the peptides are structurally unique in having at least 4 residues of p- carbamoylated Glu out of 17 to 27 amino acids. At present, 12 natural and synthetic conantokin analogs have been synthesized. A new peptide (conantokin R) with a definite structural homology to conantokin G has been characterized from Conus radiatus venom. Although only 7/27 amino acids are identical to the other conantokins, it has the conserved sequence Gly-Glu-Gia-Gla at the amino terminus. Comparison of the biological activities of several conantokins suggests that different NM receptor targets may be responsible for the sleep and hyperactivity symptoms. Synthetic conantokin analogs indicate that Gly at position 1 and Glu at position 2 are necessary for full potency; substitution of Glu by a Gin or an L-Asp inactivated the peptide and the D-Asp2 analog produced an effect only in old mice at high dose. [Ser]4 conantokin G apparently has a normal sleep activity in young mice but it is relatively inactive in older mice. Thus the conantokin may be useful probes for changes in NM receptor subtypes as a function of development. (Supported by GM 22737 and funds from the Marine Science Institute, U. P. , J. F. R.).

DISTRIBUTION OF NM RECEPTORS ON HIPPOCAMPAL NEURONS. G.T. Jones*, J.F. McGurk, M.V.L. Bennett, R.S. Zukin, G. Collard, T. Baskett, K. L. Angelides, Department of Physiology, Baylor College of Medicine, Houston, Texas, National Institute of Neurosciences, Albert Einstein College of Medicine, Bronx, N. Y., Department of Pharmacology, Bristol University, UK.

Excitatory amino acid transmission in the CNS depends critically on the activation of ligand-gated ion channel receptors classified by their preference for the agonists kainate, quosutolate and N-methyl-D-aspartate (NMDA). The NMDA receptor has aroused considerable interest due to its unique ability to translocate the second messenger calcium and its voltage dependent blockade by magnesium ions. The distribution of NMDA receptors on the neuronal cell surface we prepared several fluorescent and biotinylated analogs of Conantokin G a polypeptide isolated from the marine snail Conus geographus to the distribution of NMDA currents in Xenopus oocytes injected with rat brain mRNA and in hippocampal slices. NMDA receptors were mapped on cultured hippocampal neurons by colloidial gold decoration and circular dietyanid microscopy and their locations resolved by immunocytochemistry using antibodies to MAP-2 and synapsin, markers of dendrites and presynaptic terminals, respectively. Labeling was present on cell bodies and dendrites, was non-uniform, and was distinct from voltage-dependent calcium channel distribution labeled by w-conotoxin. Using these fluorescent and biotinylated probes we are addressing how NMDA receptors are expressed, targeted and maintained during neuronal development. Visualization of NMDA receptors on live neurons will be a central feature in analysis of receptor distribution during synaptic plasticity. (Supported by the NIH, MRC, and Epilepsy Foundation of America.)

IN VITRO AND EX VIVO BINDING OF H-FTCP: A TOOL TO STUDY NM RECEPTOR COMPLEX IN VIVO. C. Peraquin, A. Gobelt, B. Costa, K. Rice*, B. DeCorta, B. S. Miletich and G. D. Chio. Neurosciencesl, NINDS, Laboratory of Neurosciences, NIH, Bethesda, MD 20014.

Fluoroethenylcyclopropenyl (FCP), a derivative of TCP, includes a Fluorine atom and might be suitable for PET studies as a marker for NMDA sensitive glutamate-gated currents. In vitro, the specific binding of H-FTCP to rat and mouse brain membranes was measured using the non-specific binding in the presence of 10 nM MK-801 or FCP from the total binding. For ex vivo studies, 3 nM/kg of H-FTCP (p. a. 14 C/mmol) was injected i. v. in mice and rats. The animals were sacrificed at different times and radioactive aspartic acid extracts from various brain areas were counted before and after separation on se-pak C18 cartridges. H-FTCP is metabolized in vivo to hydroxylated derivatives, with half lives of 7 min in mice and 15 min in rats. In vivo regions the specific binding of H-FTCP ex vivo was calculated 10 min after the i. v. injection by subtracting the amount of H-FTCP in animals treated with 1 mJ MK-801 from the amount of H-FTCP present in the control animals. H-FTCP specific binding is saturable in vitro and ex vivo, and the density of binding sites is highest in hippocampus, followed by cerebellar cortex, and striatum; and lowest density is in cerebral cortex. Drugs acting on the PCP site of the NMDA receptor (MK-801, TCP, PCP) can displace H-FTCP binding with non-NMDA selective agonists (Baclofen, D-Asp, 2-diamino-5-pyridine), a specific ligand of sigma receptors, is active only at micromolar concentration. H-FTCP binding is increased by about three fold in the presence of glutamate in vitro, and NMDA facilitation occurs to occur in cerebellum. These data suggest that FCP is a suitable ligand for functional studies of NM receptors by PET scanning technique.
39.6 MOLECULAR CLONING OF A cDNA ENCODING A KAINIC ACID RECEPTOR SUBUNIT EXPRESSED IN HUMAN BRAIN AND RETINA. Tom Stroman, David Ogida, David Weiner, Allan Levey and Mark Bassell. National Institute of Mental Health and Receptor Genetics, Inc., Bldg 36, Rm. 3D-02, Bethesda, MD 20892

Recently, a cDNA encoding a subunit of a kainic acid receptor was isolated from rat brain by expression cloning (Hollman et al., Nature 1989). We have used oligonucleotide probes derived from the published rat nucleic acid sequence to screen human hippocampal and retinal cDNA libraries (Kollman et al., submitted). Two 3 kb cDNAs (one from the hippocampal library and one from the retinal library) which appear to encode the human homologue of the published rat cDNA have been isolated. The retinal cDNA encodes the complete coding sequence of a protein that shares 97% amino acid homology with the rat kainic acid receptor subunit.

Oligonucleotide probes derived from the human sequence encoding the putative intracellular domain have been used to localize mRNA in the middle frontal gyrus, hippocampus, neostriatum, thalamus, midbrain, pons and cerebellum of human brain. The strongest hybridization signals were observed in the cerebellar cortex and the hippocampal formation, including the dentate gyrus and fields CA1 to CA4. Moderate signals were observed in cerebral cortex, the pontine nuclei and the substantia nigra. We are currently pursuing the detailed cellular mapping of the mRNA in human brain and retina as well as the functional expression of the receptor subunit in mammalian cells.

39.6.1 SIGMA LIGANDS POTENTIATE NMDA-INDUCED HIPPOCAMPAL NEURON ACTIVATION. G. Debonnel, P.E. Monnet and C. de Montigny. Neurobiological Psychiatry Unit, McGill University, Montreal, Quebec, Canada.

We have previously reported that low doses of the sigma (6) ligand DTG selectively potentiate NMDA-induced activation of hippocampal pyramidal neurons and that this effect of DTG is blocked by haloperidol, a high affinity sigma antagonist (Debonnel et al., 15: 133-138, 1989). It was suggested that a major role of DTG might be to modulate the NMDA response. To further verify this hypothesis, we studied the effects of other selective sigma ligands and of a structural analog of DTG devoid of affinity for sigma sites, on neuronal reactivity to excitatory amino acids in male Sprague-Dawley rats under urethane anesthesia. Five-barrelled microdialyses were used for extracellular recording of CA1 dorsal hippocampus pyramidal neurons and microdialysis of NMDA, quisqualate and kainate.

Similar to DTG, low doses (1-10 µg/kg, i.v.) of (3)pentazocine, JO-1784 (3)-N-cyclopropyl-4-[(3S)-N-methyl-N-1,4-diphenyl-1-ethoxy-3-ylamino]-benzene, BD-737 (3)-3-cis-4N-methyl-N-3,4-dichlorophenyl)-ethyl-2-(1-phenyl)cylohexylamine) and AgD11 (1-(4-aminophenyl)-1-adamantyl) markedly and selectively potentiated the excitatory effect of NMDA in a dose-dependent manner. At low doses (1-10 µg/kg, i.v.) similarly to haloperidol, BMI-14802 and (3)PPP did not have any effect on NMDA-induced activation, but dose-dependently reversed the potentiation of NMDA by a low dose of DTG. At doses up to 100 µg/kg, i.v., 2-amino-5-propinol hydrobromide, a structural analog of DTG without affinity for sigma sites failed to produce an effect on NMDA-induced activation.

These data bring additional support to the notion that an important function of sigma receptors might be to modulate the NMDA response. Furthermore, they suggest the existence of two classes of ligands: DTG-like compounds acting as "agonists" and haloperidol-like ligands as "antagonists" at sigma sites.

39.6.2 COOPERATING DNA BINDING PROTEINS AS NUCLEAR THIRD MESSENGERS IN PRIMARY CULTURES OF NEURONS. A.M. Szekely, R.E. Paulson, P. Costa, and D.R. Grayson. FGIN, Georgetown University, Washington, D.C. 20007

Coordinated changes in neuronal gene expression associated with NMDA sensitive glutamate receptor stimulation may be a key mechanism underlying long-term adaptive modifications. In primary cultures of rat cerebellar granule cells brief stimulation of this receptor resulted in a programmed induction of early response genes involving c-fos, c-jun, jun-B and zif/268. These genes encode nuclear proteins, which by interacting with regulatory DNA elements, function as third messengers bringing about the coordinated regulation of target gene expression. Using gel shift assays and western blot analysis we show that following a brief glutamate pulse, the various Fox/Jun protein complexes (hetero- and/or homodimers) display a biphasic binding to A1 DNA sequences. The composition of these complexes changes with time. The glutamate stimulation also results in an increased DNA binding activity of the zif/268 "zinc finger" protein to its consensus recognition sequence, which is structurally unrelated to the AP-1 site, and is found upstream of several early response genes. This increased activity shows a delayed appearance compared to that of the AP-1 complex. These results, in light of our further data that different stimulus-specific patterns of early gene expression exist in these cells, prompt us to elucidate the mechanism whereby these nuclear third messengers interact and mediate a temporally flexible but precise response to transmitter receptor stimulation in neurons.

39.6.10 THE HIGH AFFINITY NON-NMDA-COUPLED PCP-1 RECEPTOR IS REGULATED BY GLUTAMINE NUCLEOTIDES. Y. Itzhak, J. Stein and C. Kassem.* REPSIDNO Labs. Dep. of Biochemistry & Mol. Biology, University of Miami School of Medicine, Miami, FL 33101.

We have previously reported that the potent phencyclidine analog, (5H-1-(3-hydroxyphenyl)-1-cyclohexyl) pipеридиная (3H)PCP-3-OH) labels a high affinity binding site which is not coupled to the NMDA ion channel complex, sensitive to N-methyl-d-aspartate (NMDA) receptors, and insensitive to haloperidol (Neurosci. Lett. 104: 314-319, 1989). Since this high affinity binding site is distinct from the PCP/NMDA receptor complex and the kainic/haploidal sensitive binding site, we have further attempted to characterize this site. Specific binding of (3H)PCP-3-OH (0.6 mM) to rat cerebral cortical membranes is reduced (20-65%) in a concentration dependent manner in the presence of Gpp(NH)p (0.05-1mM). However, binding of MK 801 to the PCP/NMDA receptor complex is not affected under the same conditions. In the absence of Gpp(NH)p homogenous competition of [3H]PCP-3-OH with unlabeled PCP-3-OH resulted in the best fit for a two site model, whereas in the presence of the nucleotide only one site was detected. Our studies also indicated that in the presence of Gpp(NH)p the PCP-1 binding site is not converted to the PCP/NMDA receptor complex, suggesting that the GTP-sensitive PCP-1 receptor site represent a distinct receptor domain.
397.1 FACTORS INVOLVED IN THE REGULATION OF β- AND δ-ADRENERGIC RECEPTOR mRNA IN RAT CG3 GLIOMA CELLS: C. Hough and P.-M. Chiang. Biological Psychiatry Branch, NIMH, Bethesda MD 20892

Exposure of CG3 cells to β-adrenergic agonist, isoproterenol, leads to down-regulation of β-AR mRNA species for both β-AR and δ-AR-adenosine receptors (β-AR) within 4 hours. The mechanism of down-regulation for the two receptor subtypes differs, however, in that β-AR mRNA is simply decayed to 20% of protein levels, whereas δ-AR mRNA is transiently up-regulated before decaying to 40% of pretreatment levels. Both modes of regulation induced by isoproterenol are inhibited by β-AR antagonists, alprenolol, propranolol, beta, and ICI 118 551. Alone, these antagonists can have effects on β-AR mRNA levels similar, albeit less potent, to those of regulation which can be mimicked at slower rates by agents that can raise intracellular cAMP, forskolin, cholinergic agonists, and β-AR Ag. In the presence of protein synthesis inhibitor, cycloheximide (10 μg/ml), both β- and δ-AR mRNA decay at approximately the same rate (t½=30 min), which is not significantly changed by isoproterenol treatment. We demonstrate here the existence of two GAD genes which encode proteins that differ in molecular size, response to cAMP, and subcellular location. Further, one of these two GAD mRNAs is present in the neuronal cells of the brain, while the other is present in the sympathetic ganglia. These results suggest that regulation of β-AR mRNA occurs at the levels of transcription and translation.


In the adult rat brain the major exon for Glutamic acid decarboxylase (GAD) is expressed as a 3.7 kb transcript coding for a 67 kDa protein. An alternative form of the GAD transcript was detected in the brain by nucleic acid protection. (Bond et. al. PNAS 85:3231, 1988). The structure of this alternative form is reported here. A segment of the GAD mRNA from fetal to adult rat brain was sequenced and analyzed by PCR. The sequence consists of a known adult structure with an additional 66 bp exon. Conceptual translation of the novel structure showed a stop codon in the open reading frame of the message and predicts a truncated protein product of 25 kDa. This stop codon is 5' to the pyridoxal phosphate binding site, therefore the predicted truncated protein cannot function as a decarboxylase. Genomic clones with the GAD gene have been isolated. One of these clones contains a 66 bp exon flanked by intronic sequences. To determine the level of expression of the embryonic exon, protection assays were done utilizing this transcript. These assays show that the embryonic fragment is present throughout the embryonic brain but not in the adult. These data suggest that the appearance of functional GAD requires two types of control. First, transcription must be initiated. Next, the splicing machinery must be regulated so as to eliminate the stop codon containing exon from the mRNA so as to produce functional GAD.

397.3 TWO GENES ENCODE DISTINCT GLUTAMATE DECARBOXYLASEx WITH DIFFERENT RESPONSES TO PYRIDOXAL PHOSPHATE: N.G. Erlander, N.K. Tillikainen, E. Feldblum, R. Fzal, and A.J. Tobin. Department of Biology, UCLA, Los Angeles, California 90024

Gamma-aminobutyric acid (GABA) is unmatched by any other known inhibitory neurotransmitter in its almost ubiquitous distribution in vertebrate brain. In adult brain, the synthesis of GABA depends on the activity of glutamic acid decarboxylase (GAD; E.C.4.1.1.15). The mammalian brain contains at least two forms of GAD, which differ from each other in size and in interaction with the cofactor pyridoxal-5'-phosphate (P5P). This report answers a fundamental question posed since the early 1970s: is the observed heterogeneity of the result of more than one GAD protein by electrophoresis. We demonstrate here the existence of two GAD genes which encode proteins that differ in molecular size, response to cAMP, and subcellular location. Further, one of these two GAD mRNAs is present in the neuronal cells of the brain, while the other is present in the sympathetic ganglia. These results suggest that regulation of β-AR mRNA occurs at the levels of transcription and translation.


Dopamine β-hydroxylase (DBH), which catalyzes the conversion of dopamine to norepinephrine, is regulated by various hormonal and neuronal factors. To study the molecular mechanisms of this regulation, we examined the effects of glucocorticoids and growth factors on DBH mRNA in PC12 pheochromocytoma cells.

Dexamethasone (1 μM) increased both the 2.7 and 2.5 kb mRNAs for DBH about 10-fold. This increase was dose-dependent and occurred by 7 hrs and maximal at 24 hrs of treatment. Treatment with 8-bromo-cAMP (1 μM), or forskolin (10 μM) also elevated both mRNAs for DBH with maximal induction at 4 hrs of 25-30% above control. DBH mRNA remained elevated for 1 day, but with longer exposures decreased to below control levels. Combined treatment with 8-bromo-cAMP and dexamethasone gave results similar to 8-bromo-cAMP alone. The mRNA levels for tyrosine hydroxylase (TH) essentially paralleled the induction of DBH mRNA with either dexamethasone or cyclic AMP. Nerve growth factor, in contrast, decreased both DBH and TH mRNA levels.

The results of this study demonstrate that DBH mRNA is subject to regulation by several physiologically important factors. Genomic clones to rat DBH have been isolated to begin to identify regulatory elements.

397.5 COMBINED NIOITIC AND MUSCARINIC REGULATION of CO-LOCALIZED ADRENERGIC TRANSMITTER GENES: PREPREGNEKPHALIN (ppENK) and TyROSINE Hydroxylase (TH): J.D. DeCristofaro, G. Veltinger and R. La Gamma. Dept. of Pediatrics, SUNY at Stony Brook, NY 11794-8111.

Combined nicotinic and muscarinic stimulation results in a greater than additive rise in steady state ppENK mRNA, enkephalin prohormone and peptide (Neuroscience 35:203, 1999). The rise in ppENK is 50-fold after 72 and 100-fold after 4 days of treatment. Steady state TH mRNA peaks at 72 and plateau at 10-fold over baseline. Both ppENK mRNA and TH mRNA levels remain constant over 1 week following the 4 day treatments. This suggests independent regulation of these co-localized messengers. To determine the contribution of transcriptional induction on this rise and fall in steady state ppENK mRNA, SI and primer extension analysis were performed. The greatest rise in ppENK RNA occurred 2 days after combined cholinergic treatment. By week after completion of 4 day treatments, three of the four start sites identified by primer extension analysis were no longer detectable. The data suggest that cholinergic induced initiation of ppENK RNA transcripts contributes to the rise in steady state mRNA. Moreover, these changes may play a role since initiation decreases after 2 days of treatment, but RNA levels continue to rise up to 4 days. Supported by NSP #NS057162.

397.6 TYROSINE HYDROXYLASE (TH) GENE EXPRESSION IN BOVINE ADRENAL CHROMAFFIN CELLS: REGULATION BY CHOLINERGIC RECEPTOR STIMULATION: N. Olama, M. Memo, D. Drayson, A. Goldetti and E. Costa. FGU, Georgetown University, Washington, D.C. 20007.

Cyclic AMP-mediated gene expression in adrenal chromaffin (AC) cells is transynaptically regulated by neurotransmitters released from the splanchic nerve terminals (Biochem. Pharmacol. 26 (1977) 817-823). Acetylcholine (ACh) has been released from the splanchic nerve. Primary cultures (5-7 days at in vivo) of bovine AC cells were utilized as a model system. Exposure of the AC cells to 0.1-1.0 mM carbachol slightly (ca. 20%) increased the TH mRNA when measured 48 hrs after the beginning of the treatment. However, if carbachol was applied together with HL-725 (Trequelin, 10 μM), a type II phosphodiesterase (PDE) inhibitor (Boehringer-Mannheim, Inc.), the increase was enhanced (ca. 70%). The facilitatory effect of HL-725 confirmed the involvement of cAMP in the transduction regulating the level of TH mRNA (Mol. Pharmacol. 19 (1979) 855-876). Since in splanchic nerve terminals ACh interacts with VIP, which is known to utilize cAMP for its signal transduction, the possibility that VIP functions as a co-transmitter with ACh in the transynaptic induction of TH was investigated. VIP (10 nM) produced a dose and time dependent increase of TH mRNA in absence of HL-725. More, the increase was observed only in the presence of HL-725. However, the low dose facilitated the action of carbachol. The co-stimulation of the embryonic exon, 10 μM VIP together with HL-725 increased the TH mRNA content in a time-dependent fashion.
397.7 TIME DEPENDENT REGULATION OF TYROSINE HYDROXYLASE mRNA IN S. NIGRA DOPAMINERGIC NEURONS FOLLOWING 6-OHDA LESION. G.M. Patierno, L.F. Reinhard, A.L. Kelly, D.G. Morgan and C.E. Finch, Andrus Gerontology Center and Dept. of Biological Sciences, Univ. of Southern California, Los Angeles, CA, 90009-0741. The Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Long term nigral 6-hydroxydopamine (6-OHDA) lesions induce atrophic changes in the nigral, s.nigra dopaminergic (TH immunopositive) neurons, including >50% loss of TH-mRNA concentration per neuron [Patierno et al., Mol. Brain Res. 5:203 (1989)]. We extended these studies by examining the time course of changes in the remaining s. nigra DAergic neurons at 221, 290 and 270 days following nigral 6-OHDA lesions. TH enzymatic activity, TH post-synaptic content, dopamine (DA) and DA catabolites (HVA and DOPAC) at the striatal terminals, were measured to the multiple levels of DAergic regulation in response to nigral lesioning. Nigral DAergic change began and lasted as 90 days post lesion as indicated by a reduction of nigral DAergic perikaryal area (>20%) and decreased TH-mRNA concentration (>30%) per neuron. The decreased TH-mRNA concentration at 270 days confirms previous results. In contrast to these atrophic nigral changes, compensatory changes were observed in striatum. At 21 days post lesion, the striatal DA content, HVA, was reduced slightly less than TH catalytic activity, which suggests increased (>30%) striatal DA release and utilization. Because the loss of striatal TH polypeptide content was less than the loss of DAergic neurons at all times after nigral 6-OHDA lesions, it follows that the surviving s. nigra DAergic neurons compensated by increased production of TH protein. Thus, the surviving neurons become more efficient in utilizing the available TH-mRNA with regard to the final product, striatal TH polypeptide. (Supported by National Parkinson Foundation and United Parkinson Foundation to CEF and GMP and by John D. and Katherine T. MacArthur Foundation Research Program on Successful Aging to CEF. DOM is an Established Investigator of Amer. Heart Assn.).

397.8 LOCAL PROTEIN SYNTHESIS WITHIN ISOLATED GROWTH CONES OF CULTURED SNAIL NEURONS. Laura Davis and Stanley B. Katsumi, The Program in Neuronal Growth and Development, Center for Developmental Biology and Anatomy, Colorado State University, Fort Collins, CO 80523.

The neuronal growth cone is a highly specialized region at the tip of growing neurites that contains a complex organization that is different from the neuron perikaryon, and an intracellular calcium concentration that is apparently independently regulated. It has been suggested that growth cones may function independently from the rest of the neuron and, in fact, the behavior of isolated growth cones is indistinguishable from that of intact for at least 48 hours. Further autonomy would be conferred by the ability to synthesize new localized proteins. To explore the possibility that proteins are locally synthesized in growth cone, cultures of Helisoma neurites were prepared and the growth cones of 1-2 neurites per cell were transplanted. Two cultures were pulse labelled in 3H-leucine for 10 minutes and then fixed and processed for autoradiography. Autoradiographs demonstrated that isolated growth cones was comparable to labeling over growth cones and distal neurites whose cytoplasm was continuous with the rest of the cell. Because small neurites are impermeable to most conventional protein synthesis inhibitors, we employed DMSO (8%), a reversible inhibitor of ribosomal protein synthesis, at a concentration that pulse-treated neurones survived and continued to grow for at least 6 days. Labelling was greatly reduced or eliminated over neurons, as well as isolated growth cones, in DMSO treated cultures; a control that suggests that 3H-leucine is indeed incorporated into newly synthesized proteins during translation. These results suggest that proteins are locally synthesized in isolated neuronal growth cones. This may indicate that the expression of the genes can be regulated independently by individual subcellular regions. Thus, the complement of protein complexes within a growth cone might be modified in response to local stimuli. Supported by NIH NS8445.

399.1 TOWARDS A NEUROBIOLOGICAL THEORY OF VISUAL AWARENESS. G. Koch and F. Crick, CNS Program 216-76, Caltech, Pasadena, CA 91125 and Saik Institute, La Jolla, CA 92037.

We propose a neurobiological framework to study one particular form of consciousness: visual awareness. We postulate one form of awareness linked to the serial, attentional mechanism studied by Julesz, Treisman and others. This working awareness (WA) solves the binding problem by temporarily integrating activity in any two points in the visual field - the key feature of the Gestalt. WA allows a person to solve a problem by temporarily integrating activity in different parts of the visual field. Thus, we propose that visual awareness is related to the temporary integration of different aspects of the visual field. This theory is consistent with the idea that the visual field is composed of a set of 'modules' that can be temporarily integrated to form a single 'unit'. We hypothesize that this process is mediated by a set of 'modules' that can be temporarily integrated to form a single 'unit'. We hypothesize that this process is mediated by a set of 'modules' that can be temporarily integrated to form a single 'unit'. We hypothesize that this process is mediated by a set of 'modules' that can be temporarily integrated to form a single 'unit'. We hypothesize that this process is mediated by a set of 'modules' that can be temporarily integrated to form a single 'unit'. We hypothesize that this process is mediated by a set of 'modules' that can be temporarily integrated to form a single 'unit'. We hypothesize that this process is mediated by a set of 'modules' that can be temporarily integrated to form a single 'unit'. We hypothesize that this process is mediated by a set of 'modules' that can be temporarily integrated to form a single 'unit'. We hypothesize that this process is mediated by a set of 'modules' that can be temporarily integrated to form a single 'unit'. We hypothesize that this process is mediated by a set of 'modules' that can be temporarily integrated to form a single 'unit'. We hypothesize that this process is mediated by a set of 'modules' that can be temporarily integrated to form a single 'unit'. We hypothesize that this process is mediated by a set of 'modules' that can be temporarily integrated to form a single 'unit'. We hypothesize that this process is mediated by a set of 'modules' that can be temporarily integrated to form a single 'unit'.
398.4
DOES MOTION PERCEPTION DEPEND ON THE MAGNOCELLULAR PATHWAY? William H. Merigan, Carey Byrne, and John Maunsell, University of Rochester, N.Y. 14622.

There is some evidence that visual signals relayed through magnocellular layers of the lateral geniculate (M pathway) may be particularly important for motion processing. Anatomical and physiological data indicate that this pathway provides the major input to the cortical "motion" pathway, which includes areas MT and MST. A recent lesion study has shown that damage to the M pathway disrupted motion detection, whereas damage to the P pathway had no such effect (Schiller et al., 1980).

398.5
CORTICAL DYNAMICS OF VISUAL MOTION PERCEPTION: SHORT- AND LONG-RANGE APPARENT MOTION. Michael E. Rudd and Stephen Grossberg, Center for Adaptive Systems, Boston University, 111 Cumnnington Street, Boston, MA 02215.

Further development of a neural network model of motion perception by visual cortex (Grossberg and Rudd, 1985) is presented. The model clarifies computational differences between parallel cortical streams for perception of static form (V1 → V2 → V4) and moving form (V1 → MT → V2 → IT). Properties of the model are tested by simulation of psychological data concerning two-flash and Ternus apparent motion displays, as well as percepts of split motion, reverse motion, gamma motion, and interactions between continuous motion and band color percepts. The model explains the apparent space-time separability of the motion strength function as well as deviations from space-time separability at threshold. The model clarifies the dependence of the maximum extent of short-range motion on element scale, and the different properties of short-range and long-range motion vis-à-vis interferance changes in stimulus direction-of-contrast. In addition, the model clarifies how sustained and transient channels cooperate and compete in successive processing stages to generate motion signals that are independent of direction-of-contrast; and how pre-processing of motion signals by a Motion Oriented Contrast (MOC) Filter is joined to long-range cooperative-competitive feedback mechanisms, called a CC Loop, to control phenomena such as induced motion and motion capture.


398.6

Investigations in depth perception using disparity information have demonstrated surface interpolation between feature elements. Current computational algorithms of structure from motion (SFM) do not perform such an operation. Here we present strong evidence for surface interpolation in SFM and in the following abstract demonstrate how such a mechanism can be incorporated into current computational algorithms. Using the parallel projection of randomly positioned dots on a rotating transparent cylinder, we impose a desynchronized oscillatory motion on the individual dots. Although we do not have a rigid physical correlare, subjects perceive a rigid cylinder in smooth rotation. This percept can only be explained if the visual system represents the observed object not as a collection of individual elements but as two surfaces moving in opposite directions. A given point contributes information to one of the surfaces depending on its direction.

The basis for this segmentation of surfaces might be direction selective cells in V1 which act as directional filters ignoring motion in the non-preferred direction (Erickson et al. Neurosci. Abstract, 1989). In a population of such cells our stimulus would activate two distinct groups of cells and an oscillating dot would activate either alone or the other group of cells.

398.7

We present a model for the human recovery of 3-D structure from motion that combines the abstraction of Illi's incremental rigidity scheme with a surface interpolation process that reconstructs full 3-D surfaces from sparse depth information. The input to this algorithm consists of either the velocities or displacements of moving points in the image. Using this information, the algorithm sequentially estimates a new 3-D structure by minimizing the overall deviation from rigidity. As 3-D structure is derived at the locations of the moving points, a smooth surface is filled in between these locations. Since what is preserved between views is the reconstructed surface, individual image features may appear and disappear without destroying the recovered structure.

This model allows multiple surfaces to be represented simultaneously in order to cope with transparency, facilitates the use of constraints on surface shape from object boundaries, and groups points on different surfaces based on their 2-D image motion. Thus the model can account for experimental observations by Hussain et al. (Neural Comp., 1:324-333, 1989) and Tread et al. (preceding abstract) regarding the ability of human subjects to interpret 3-D structure in displays with limited lifetimes. We show that this model can account for many other experimental observations such as general degradation of performance with fewer points, as well as the interactions between multiple transparent surfaces in motion suggested by Ramachandran et al. (Perception & Psychophys, 44:330-393, 1989).

398.8
LESIONS OF AREA 17 IN THE CAT REDUCE SENSITIVITY TO HIGH BUT NOT LOW SPATIAL FREQUENCY TARGETS. Tatiana Pasternak, luana Zarnowska, and John H. R. Maunsell, Dept. of Neurobiology and Anatomgy, Physiology, and Center for Visual Science, University of Rochester, Rochester, NY 14627.

We have recently shown that binocular lesions of area 17 severely reduce the sensitivity of cats to drifting low spatial frequencies, but leave the sensitivity to high spatial frequencies intact (Pasternak et al., 1989). This result is consistent with the physiological properties of neurons in area 17, which on average prefer targets of spatial frequencies three times lower than neurons in area 17. The intact acuity fol- lowing lesions of area 18, and the high spatial frequency preferences of area 17 neurons suggest that the detection of high spatial frequencies may depend upon neurons in area 17. To assess the contribution of area 17 to spatial vision we placed punctate stimuli, occurring in addition, a physiologically identified portion of area 17. Each lesion was centered at an eccentricity of about 8° along the horizontal meridian of the left visual field. We measured the detectability of various spatiotemporal targets placed within the ablated portion of the visual field representations, while monitoring eye position with a scleral search coil. The cats were required to maintain fixation on a laser spot and respond to the presence or the absence of the grating by press- ing a right or left pedal. We found a loss (30%) of visual acuity for targets placed within the lesioned part of the visual field. Furthermore, contrast sensitivity for stationary gratings of intermediate and higher spatial frequencies was reduced by about a factor of 2. On the other hand, contrast sensitiv- ity was normal at low spatial frequencies (0.28 c/deg). The results were similar for grating drifts at 4.5 Hz. This result, in conjunction with previ- ously reported deficits following low contrast temporal luminance signals, demonstrate that high level functional segregation at early stages of cortical processing, with area 17 playing an important role in spatial resolution, while area 18 contrib- utes to the detection of low spatial frequencies. Supported by EY06175, EY05319, and EY05911.
398.9


We have investigated the effect of large extrastrate lesions (Vandebusche et al., Soc. Neurosci. Abstr. 15:1255, 1989) on orientation (O) in two cats (52 and 56), trained in O of a bar and of two types of illusory contours. The length of illusory contours and bar was 12 cm. The contours used were Gap Illusory Contour (GIC) - 7 semicircles separated by 1.2 degree wide gap - and Phase Shifted Illusory Contour (PSIC) - 4 semi-circles after 1.2 degree contour. 75% correct Just Noticeable Differences (JNDs) were determined for each contour-type in an interleaved way, presenting one contour-type only at a time. Although we have no histological control of the lesion yet, it was intended to destroy areas 15, 20 and 21, and medial bank of the Lateral Suprasylvian Sulcus. The two cats behaved very similarly during pre- and postoperative testing. Average preoperational JNDs were 5.8, 11.1 and 15.9 degrees for bar, GIC, and PSIC respectively. During the first postoperative month, no reliable thresholds could be measured for the PSIC, whereas thresholds for the bar and the GIC were 11.8 and 33.4 degrees respectively. After three months JNDs stabilized at 7.5, 23.0 and 38.3 degrees. These results suggest that extrastrate areas are more critical for O of illusory contours than for O of bars.

398.11

LONGITUDINAL DEVELOPMENT OF EYE ALIGNMENT IN KITTENS WITH TWO TYPES OF SURGICALLY INDUCED STRABISMUS: RELATIONSHIP TO SURGICAL CORRECTIVE BINOULARITY by Xiaoxia Vriend*, W. Singer, M. Fraunius*, Jochen Greuel and Johannes Post*. Max-Planck-Institute for Brain Research, Frankfurt/Main, FRG.

Interocular alignment was assessed by corneal light reflex photography in 15 normal and 24 strabismic kittens. Nineteen strabismic and 5 normal kittens were followed longitudinally from 10 days to 12 months of age. Strabismus was induced at 3-4 weeks of age, either by cutting one extraocular muscle (tenotomy) or by reinserting a muscle at another position on the ocular globe (recession).

Four out of the 6 tenotomized cats and 5 out of the 11 recessed cats showed a post-operative ocular deviation throughout the testing period ("large-angle strabismus"). Two tenotomized and 6 recessed cats showed a transient deviation for 1-2 weeks after surgery, which the interocular alignment stabilized to values found in normal cats ("microstrabismic"). All cats showed a clear break down of binocularity in area 17. In the postero-medial lateral suprasylvian area (PLS), binocularly was markedly reduced for the large-angle strabismics, but much less so for the microstrabismic cats. The difference might be due to the coarse grain and poor retinotopic organization of visual receptive fields in the lateral suprasylvian area.

398.12

SIGNAL AND MESSAGE IN DIGITIZED SMILES. C.M. Leonard, Dept. of Neuroscience, Univ. FL, College of Medicine, Gainesville, FL 32601.

One of the most complex information processing tasks a human can perform is the decoding of social communication signals such as facial expressions. There are few methods presently available for measuring information content in these signals. This abstract describes the information generated by mouth movements in digitized human smiles. Facial movements were measured by digitally subtracting adjacent frames from 800-msec taged vignettes (12 frames) and calculating the variance of the pixel values in the subtracted images. The eyes were held constant in all frames. Twelve observers were asked to indicate the social message perceived in individual images in each vignette. The total number of positive messages assigned to each smile correlated .74 (Spearmann r, p < .05) with its cumulative variance. In five of the smiles, the perception of positive messages increased during 100-msec periods of high variance associated with a deepening frown on the right side.

In order to measure the relation between changes in signal and perceived message for individual observers, a sequential, two alternative, forced-choice psychophysical procedure was used. The stimuli were brief (200-msec) two-frame sequences presented forwards (increasing smile) and backwards (decreasing smile) in pseudo-random order on each trial. Subjects were asked to report whether the first or second second of images was more pleasant. The strength of the preference for the forward order ranged from 50 to 100% for different sequences and depended on the between-frame variance. Rapidly increasing smiles (those with large variances) were more reliably perceived as pleasant than gradually increasing ones. These results demonstrate that digital image analysis and psychophysics can be used to identify stimulus characteristics that define the boundaries between social messages in a rapidly changing signal. This method permits quantification of the hedonic qualities of facial expression.

TRANSPLANTATION: NEW TECHNIQUES, IMMUNE REJECTION AND BEHAVIOR

398.2


The ability to maintain in storage a pool of a specific neuronal population would be important in brain transplantation. We have observed (Gapper et al., Soc. Neurosci. Abst. 15:708, 1989) that exposure of rat mesencephalonic cell cultures to epidermal growth factor (EGF) results in cell proliferation and confluence within 3 weeks in vitro. Staining with GFAF indicates that only a fraction of the cells are differentiated astrocytes. We considered the possibility that the apparently undifferentiated cells were both neuronal and glial precursor cells. We showed that survival and dissociation and differentiation after replanting. Mesencephalonic cells from 6-10 rat embryos were grown in chemically defined medium with 12.14 GFAF. After six days the replanted were removed from the dishes by 0.064 trypsin, washed and resuspended in medium by trituration. 5x10⁶ cells were plated on polystyrolite coated 35mm dishes in 1 ml of medium with 10% FBS. Cells were allowed to adhere on the dish and phase bright cells attached to the dishes within 24 hrs and a large number of the phase bright began extending processes. Lesions of 3-5 days after replanting, cultures were stained with antibodies to GFAF or Tau to determine the presence of differentiated astrocytes and neurons. Most of the flat cells, about 20% of the total cell population, were positive for GFAF. A number of the conserved PC12 cells were positive for Tau, had extended long processes and had the appearance of well differentiated neurons. Supported by NIH grants NS-301917; 08-07245 and United Parkinson Foundation.

We have exploited the natural biology of herpes simplex virus (HSV) to engineer recombinant HSV vectors of reduced neurotoxicity. Infection of the latex core of the V58R LAT promoter was recombinant into HSV as a reporter of foreign gene expression during productive and latent viral infection respectively.

In vivo, infected Vero cells showed intranuclear viral particles, and β-gal as demonstrated by the X-gal reaction product. Efficient pLacZ recombinants expressed β-gal in the absence of detectable viral particles.

In vivo, wild-type and glycoloprotein C (gC) vector (galactosidase (β-gal) was expressed and observed in infected cells expressing β-gal in neonatal retinal transplants.

These results show that HSV vectors can be used to express foreign genes in cells of brain.

390.9 IMMUNE PRIVILEGE IS EXTENDED TO INTRAOCULAR DEVELOPING RETINAL ALLOGRAFTS. L.-Q. Jiang and J. W. Streit, Dept of Microbiology & Immunology, Univ. of Miami School of Medicine, Miami, Fl 33101.

One long experiment goal of retinal transplantation is to be able to transplant viable retinal tissue into eyes blinded by retinal degenerative disorders. The success of such grafts would be expected to depend partly upon the ability of the recipient's immune system to recognize the graft's alien transplantation antigens and mount an immune response. To examine the immune response evoked by intracranial retinal transplants, we transplanted, bioluminescent-refllected BALB/c retinas into the anterior chamber (AC) and subconjunctival (SC) space of adult C57BL6 mice. Clinical and histologic examination indicated that even strongly histoincompatible developing neural retinal transplants, engrafted within the AC, acquired a blood supply, and differentiated into recognizable retinal structures, without the evidence of rejection. SC retinal grafts were rejected. To examine whether mice with intracranial retinal allografts developed a deviant immune response, a panel of C57BL6 mice (5) received from histoincompatible developing neural retinal transplants into the AC, or in the SC. Twelve days later, the eyes of these mice were tested for delayed hypersensitivity by intraperitoneal injection of BALB/c spleen cells. A positive control panel was defined by injection of AC and SC BALB/c spleen cells. A positive control panel was defined by injection of BALB/c splenic cells into mice and into the host. No immune response was observed when injected spleen cells were derived from mice engrafted with a spleen cell implantation into the striate of lesioned rats was also evaluated. Melanocytes implanted in normal and lesioned striata survived but did not appear to proliferate when examined at various times post-implantation. Melanosome activity, the level of which was measured in melanocytes implanted in both normal and lesioned striata. However, melanosomes in the striata of lesioned rats appeared to be degenerating and lymphocyte infiltration was present. Rats with melanocytes implanted in lesioned striata showed contralateral rotation induced by the injection of apomorphine.

These data suggest that melanocytes may serve as an alternative transplantable cell source of DOPA for the Parkinson's disease. Supported in part by USPHS Grant MH 14092, NS26068 and the United Parkinson Foundation.
THURSDAY AM
TRANSPLANTATION: NEW TECHNIQUES, IMMUNE REJECTION AND BEHAVIOR

399.9

Starting three months after grafting, behavioral effects were assessed and open field, radial maze and learning of intrahippocampal grafts of fetal ED14 (Group S14) and ED16 (Group S16) septal-diagonal band tissue, or of cortical tissue, as a control group (Group C2). The grafts were assessed in Long Evans female rats given electrolytic lesions of the dorsal subcallosal septo-hippocampal pathways (Group L). Sham-operated non-grafted rats served as controls (Group D).

Compared to S14 rats, L rats showed increased activity in both the open and closed field, as well as a dramatic improvement in maze learning. Neither type of graft provided any benefit on the maze activity, but graft-induced improvement was observed in the open field scores only in S16 rats. These results, which will be discussed in the light of historical data, suggest that beneficial effects of septal grafts can be achieved by visual and implanted behavioral deficits can be expressed differentially as a function of the fetal donor’s maturity stage.

399.11
IMPLANTED INTRA STRIAL MICROENCAPSULATED DOPAMINE REDUCES DA AGONIST- INDUCED ROTATIONAL BEHAVIOR. A. McKean*, S. Hichens*, D. Mason*, L. Dillon*, A. Tae, University of Goteborg, Dept of Histology and Pharmacology, Goteborg, Sweden 40533 and Southern Research Institute, Birmingham Alabama USA 35255.

Dopamine (DA) has been encapsulated in 2 different polymeric materials (DL-lactide-co-glycolide) in a 399.12

399.10
HOMOTOPIC NEURAL GRAFTS DO NOT CONSISTENTLY IMPROVE FORELIMB REACHING PERFORMANCE OF RATS WITH EXCITOTOXIC LESIONS OF THE STRIATUM. H. Min, K. McLean and D.A. Schrag. Dept. Biomedical Sciences, McMaster University, Hamilton, Ont., Canada, L8N 3Z3.

Female Wistar rats with bilateral, quinolinic acid-induced, excitotoxic lesions of the rostrolateral striatum were assigned to groups at random for homotopic transplants of rat striatal homotopic grafts (homotypic grafts) or cortex (heterotypic grafts) or sham-operation (lesion-only group). The heterotypic grafts were either unilateral or bilateral. The reaching performance of these rats was compared with that of control rats with neither lesions nor grafts, approximately 1 year after transplantation. The lesions produced a significant improvement. The rats with either homotypic grafts or unilateral heterotypic grafts performed more poorly than the controls and did not reliably differ from the rats with lesions only. In contrast, the rats with unilateral homotypic grafts did not significantly differ from the controls. However, these rats preferentially performed the paw contralateral to the homotopic transplantation. Histology confirmed the homotypic and heterotypic grafts, but not consistently the survival and growth of all grafts. Homotopic neural grafts do not appear to benefit the reaching performance of rats with striatal lesions, at least not consistently. (Supported by the BRC of Canada, H. Min is a Research Associate of the Ontario Mental Health Foundation).

399.12
TRANSPLANTED RETINA: ASSESSMENT OF FUNCTIONAL INTERACTIONS WITH HOST OPTIC INPUT AND SENSITIVITY TO ILLUMINATION. L. DeR*, Dept. Neurobiology, Anatomy & Cell Science, Univer. Pittsburgh Sch. Med., Pittsburgh, PA 15261. We report results from studies in which retinal responses of adult rats were elicited by illumination of the host visual nerve. These studies were performed on 3 rats, and the retinatal implant was recorded over a several week period in the same animal. If one host eye is removed at the time of transplantation (e.g. postnatal day 1) an extensive transplant integration occurs, with the host visual nerve (e.g. optic nerve) remaining intact. A marked improvement in the response can, however, be observed within 24 hrs after the optic nerves are sectioned. The time course and change in the response is very similar to that seen in the transplant-implanted response. (Supported by NIH grants EY02583, EY02967 & HD07747).

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
400.1
POLYETHYLATION OF GLUCOSE UTILIZATION AND TRANSPORT IN SYNAPTOSONIC CULTURED O-2 CELLS AND ASTROCYTES. S.P. Sood-grass and M.H. Morita*, Neurology Research Laboratory, Children's Hospital, Los Angeles, and Dept. of Neurology and Pediatrics, University of Southern California, Los Angeles, CA. Polates have found polyol stimulation of \( \alpha \)-uptake, cGMP accumulation, and phosphatase transport. We studied fo- toelectrical cGMP accumulation (cGMP) in rat brain slices, astrocytes, and synaptosomes. FA and methoctramine (MDM) produced 32% and 48% increases at 10 \( \mu \)M concentra- tion (10 min incubation, 45 min incubation, effect peaked if preincubation exceeded 30 min). Higher FA and MDM con- centrations produced larger increases, up to 3 fold. The physical stimulation of cGMP accumulation (MDMP) was a weaker stimulator of cGMP. Stimulation of the part of the fo- late molecule, also causing dose dependent increases of cGMP. Polates responses were smaller in astrocytes and synaptosomes than in O-2 cells but statistically significant. Polates produced more stimulation of U-\( \alpha \)-glucose accumulation than of DPGA, and greater effects on DPGA than on 3-O-methyl glucose accumulation. Polates stimulate both glycolysis and glucose transport in several neural cells. It is interesting that cGMP and dihydroxyfolic acid also inhibited by chloromethane and other pyridines.

400.2
ACTIVATION OF ARACHIDONIC ACID METABOLISM BY PAF AND CALCIUM-IONOPHORE IN PRIMARY CULTURE OF ASTROGLIAL CELLS. A. PETROZZI, M. Blasiecy*, F. Visioli*, B. Zanocco*, G. Reccagni and C. Galli*, Institute of Pharmacological Sciences, University of Milan. Astrocytes actively metabolize arachidonic acid (AA) via the 1p6 (LO) and cytochrome pathways after appropriate stimulation. CO and LO metabolites play important roles such as cerebral ischemia, convulsions, and inflammation. Moreover, LO products have been proposed as intracellular second messengers in EPL. (Pomelli et al., Nature 339:58, 1989) and the investigators have investigated the activation of PAF and PAF, which was investigated by incubating the cells with Triton A and evaluating the radioactivity labelled metabolites. Incubation with PAF and PAF increased at different conditions and for different times. The LO products hydroxy-eicosatetraenoic acids were measured by HPLC and GC-MS. LTC4 and BPA were identified by a second messenger activation of AA metabolism by PAF and PLC. The activation of CO and LO pathways was dependent on the time and the concentration of the stimulus. In addition, AA metabolism was affected by precipitating the astroglial cells with PAF antagonists and ganglioside derivatives. This work was supported by FIDIA S.p.A., Abano Terme, Italy.

400.3
SOMATOSTATIN (SS) AUGMENTATION OF THE M-CURRENT (I\text{M}) IN HUMAN MAST-CELL MEDIANELASTIC LEUKOTRIENE, E. Schweizer*, S. Madambri and G.R. Signes. Dept. of Neuropharmacology, Scripps Clinic Research Institute, La Jolla, CA 92037.

400.4

400.5
MUSCARINIC-STIMULATED CALCIUM MOBILIZATION IS NOT COUPLED TO CYCLIC GMP GENERATION IN N1E-115 CELLS. A.B. Morrelli and S.L. Thompson. Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950.

400.6

We are investigating the mechanisms of calcium mobilization through the phosphatidyl inositol (PI) cascade. Fluorescence imaging, microperfu- sion, and test photolysis are used to observe the responses of single N1E-115 cells to the muscarinic agonist carbachol (CIC). Cells are loaded with the calcium indicator fura-2 or Fluo-3. 1 mM CaCl\textsubscript{2} is added to the back of the bath or with a rapid, focal perfusion micro- pipette. The spatiotemporally complex "finger-prints" observed as reginal calcium in drug applications 30-60' apart (bath) or 30-60' apart (microperfusion). With microperfusion, Ca transients far over the bath. Return to basal (Ca\textsuperscript{2+}) after a spritz of Ca is followed by a refractory period during which the time course of Ca mobilization is altered. We are using flash uncaging and microinjection of Ca and phospholipidol to assess the role of second-messenger cascades in Ca mobilization, by inducing step changes in PI metabolites and Ca.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

The stimulation of N-methyl-D-aspartate (NMDA) receptors in cerebellar granule cells activates a guanylate cyclase and increases cyclic GMP content. To establish whether this signal transduction is mediated through the activation of nitric oxide (NO) synthetase which generates NO, an activator of soluble guanylate cyclase, the enzyme activity was measured in various cultures of cerebellar granule cells incubated with L-H{sup 3}arginine by following its conversion to L-H{sup 4}citrulline. Activation of NMDA receptors by glutamate or NMDA increased in a dose-dependent manner the formation of L-H{sup 4}citrulline and the cyclic GMP accumulation, measured in the same samples. Both effects were blocked by the NMDA receptor antagonist MK-801 (1 mM) and the NO synthetase inhibitor N\textsuperscript{6}-methyl-L-arginine (1 mM). The activation of NO synthetase was also evoked by the calcium ionophore A23187. The maximal conversion of the radiolabeled into L-H{sup 4}citrulline occurred 5 min after the application of the agonist and then remained constant for 60 min. Further enzyme activation was not produced by subsequent additions of glutamate or the calcium ionophore. These results indicate that the activation of NMDA receptors leads to a transient increase in the activity of NO synthetase. Since cyclic GMP accumulation showed a similar transient responsiveness to glutamate, one might infer NO synthetase desensitizes.


In primary cultures of cerebellar neurons the stimulation of N-methyl-D-aspartate (NMDA) receptors leads to the activation of guanylate cyclase and cyclic GMP accumulation. This stimulation was reduced in a dose-dependent manner by N\textsuperscript{6}-methyl-L-arginine, an inhibitor of nitric oxide (NO) synthetase, indicating that cyclic GMP accumulation depends on NO-stimulated NO formation. Accordingly, cyclic GMP accumulation was induced by sodium nitroprusside (SNP), which acts by releasing NO that directly activates guanylate cyclase. The stimul of cyclic GMP formation by SNP in primary cultured cerebellar granule neurons was mediated by nitric oxide, the inhibitor of lipoygenases, indicating that the mediation of the receptor signal requires also an active metabolism of arachidonic acid. The maximal cyclic GMP accumulation induced by SNP was 3 to 4 fold higher than that elicited by NMDA, however, when both compounds were added together the effect was not additive. In fact, NMDA caused a dose-dependent inhibition of cyclic GMP formation but not its degradation. These results suggest that the NMDA receptor-induced regulation of guanylate cyclase activity may involve the interaction of two messengers, NO and a lipoygenase product of arachidonic acid metabolism.


The activation of glutamate receptors in cerebellar granule cells stimulates several signal transduction systems including the generation of nitric oxide (NO). Moreover, agents such as sodium nitroprusside (SNP), which releases NO, were shown to activate an endogenous cysolic ADP-ribosyltransferase in a variety of tissues. In order to determine whether glutamate affects endogenous ADP-ribosylation, granule cells were incubated for 20 min in the absence or presence of glutamate. They were then harvested, homogenized, and centrifuged, and the ADP-ribosylation was monitored in vitro in the presence of [F\textsuperscript{32}P]NAD in cytosolic fractions, for 30 min at 37°C and SNP, and was analyzed by SDS-PAGE. SNP caused the ADP-ribosylation of several proteins present in the cell cytosol, the most prominent being a 40 KDa band. This effect was mimicked by cyclic GMP, indicating that SNP-induced ADP-ribosylation is not mediated through the activation of guanylate cyclase, but it may be triggered by NO action. In granule cells treated with glutamate the ADP-ribosylation induced in vivo by SNP was decreased, suggesting the ability of glutamate to ADP-ribosylate the protein in intact cells, possibly through generation of NO. Similar experiments performed with cholera toxin which ADP-ribosylates specific GTP-binding proteins, showed two radioactive bands (45 and 61 KDa) present in the cell homogenates. Glutamate treatment preferentially decreased the appearance of the 61 KDa band. These results suggest that stimulation of glutamate receptors may increase either the activity of intracellular ADP-ribosyltransferases or the expression of their protein substrates, including the regulatory GTP-binding proteins.


Nitric oxide (NO) is a major, if not the sole source of endothelium derived relaxing factor (EDRF) that mediates the vasodilating effects of neurotransmitters on certain blood vessels. Cyclic GMP elevations in the cerebellum and in neuroblastoma cell lines in response to calcium-mobilizing neurotransmitters is also mediated by NO. Recently, we purified NO synthase (NOS; EC1.1.1.23) to homogeneity from rat brain (Bredt and Snyder, PNAS 82, 692-696, 1995). Our ability to purify NOS was due to the development of a simple, sensitive, and specific assay relying on conversion of [F\textsuperscript{32}H]arginine to [F\textsuperscript{32}H]citrulline, which occurs stoichiometrically with our discovery that this process requires amino pyrolysis effecting enzyme. We have raised and affinity purified two antisera which immunoprecipitate NOS activity and recognize a single 150 KDa band in western blots of numerous central and peripheral neurons. Immunohistochemistry in the brain shows NOS is discrete localized and is absolutely restricted to neurons and vascular endothelial cells. Highest density occurs in the cerebellum, cortex, and cerebellar and cortical terminals, glomerular synapses in the cerebellum, nerve terminals in the granule cell layer of the olfactory bulb, nerve processes in the posterior pituitary, and in the neuronal axons surrounding large central blood vessels. Calcium mobilization in these nerve terminals may generate NO as a unique neurotransmitter.

400.11 CHARACTERIZATION OF THE INHIBITION OF THE EAA RECEPTOR LINKED TO PI METABOLISM BY 2-AMINO-3-PHOSPHONOPROPIONATE IN THE RAT HIPPOCAMPUS. E. Palmer* and G.W. Getman, Department of Psychology, University of California, Irvine, CA 92717.

Exclusively amino acids (EAA) interact with specific membrane receptors to generate second messengers primarily PI (inositol phosphates). The most potent agonists for the PI-linked receptor are trans-1-aminocyclopentyl-1,3-dicarboxylic acid (ACPD), ibotenic acid (IBO) and quisquulate (QA). Of these, the most selective agonist for PI stimulation is ACPD. It has been reported that 1 mM L-2-amino-3-phosphonopropionate (AP3) is a very efficient inhibitor of IBO and QA stimulation of PI metabolism and that 2 amino-4-phosphonobutyric acid (L-AP4) also inhibits PI metabolism, but not as effectively. Using hippocampal slices from 8-10 day old rats we confirm that AP3 inhibits PI stimulation by IBO and QA, although lower levels of inhibition were observed previously. We further report that AP3 also inhibits the stimulation of PI metabolism by ACPD. Our data indicate that the mechanism of inhibition by AP3 is not via the A4-P receptor as inhibited by IBO, QA and AP4 induced stimulation by L-AP4 was not affected. Incubations with 1 mM AP3 performed in the presence of excess antagonist revealed that the reversal of the inhibition. Inhibition of ACPD and QA stimulation by AP3 was temperature dependent, while that of IBO was not. Thus, it appears that PI phosphorylation via a complex mechanism that is neither competitive nor reversible.


Superfused rat hippocampal mossy fiber synaptosomes were used to investigate the role of lipoygenase products in the evoked release of endogenous glutamate. Pretreatment of the synaptosomes with 12(R)- or 12(S)-HETE (10 μM) reduced the 35 S K\textsuperscript{+}-evoked glutamate release by 50% and these effects appeared to be specific for 12-HETE, since neither 5- nor 15- HETE were able to affect glutamate release. In addition, inhibition of lipoygenase by 10 μM NDBA potentiated the 35 S K\textsuperscript{+}-evoked glutamate release by 50% and the 200 μM arachidonate-induced release by 113%. The observation that 12-0-HETE inhibited the 35 S K\textsuperscript{+}-induced Ca\textsuperscript{2+} influx by 79% supports the suggestion that it modulates membrane potential and inhibits neurotransmitter-release, i.e., some of the effects with 4-aminopropylidene or 3,4-dimino propyline stimulate the release of glutamate. Therefore, it appears that 12-0-HETE acts as a second messenger to influence glutamate release from hippocampal mossy fiber terminals. The above results are consistent with the hypothesis that lipoygenase products act to activate a component which mediates an inhibition of neurotransmitter release. Supported by APOS 89-0234.
401.1 SPATIAL Response RESPONSE AND PROJECTION PATTERNS OF SECONDARY MEDIAL VESTIBULO-PULSINAL TRACT (MVST) NEURONS. S.L Perlmutter, Y. Ivanov*, J.E. Baker, B.W. Peterson, Northwestern Univ. Med. School, Chicago IL.

To determine how spatial motor patterns of the vestibulococcy reflex are produced, we studied 59 MVST neurons activated monosynaptically from the labyrinth in decerebrate cats. These neurons were identified by antidromic activation from, or by recording intraclassically in, ipsi- or contralateral C1 MVST, and by responses to electrical stimulation of IInd nucleus and the rotation produced a neuron's maximal activation was derived from its response to 0.5 Hz rotations in many vertical and horizontal planes. In some cats horse-radish peroxidase was injected intraventricularly at C1.

Neurons with different projections responded differently. Of 28 neurons projecting to IInd nucleus, 27 were not activated from C1 and had a response vector in the debracerebral canal. Vectors of neurons projecting neither to IInd nucleus nor C1 (n=19) were similar, but were often indicated considerable vertical-horizontal canal convergence. 12 of 13 neurons which did project to C1 were excited strongly by yaw rotation to the contralateral side (type II response), or had convergent input from 2 vertical canals. Four secondary MVST axons were stained with HRP. Each sent branches to a specific region of the C1 ventral horn. For example, one axon with primarily posterior canal input had dense arborization and numerous terminalis in the dorsomedial ventral horn, where motoneurons of longons can be located.

Data suggest that secondary vestibulococcy neurons primarily relay activity of a single ipsilateral semicircular canal, as do vestibulococcy neurons reported earlier. Signals were carried past puck segment.

NS17489, EYO6485, EYO5289, EY07342


The posterior region of the vestibular system is composed of two main nuclei; the medial vestibular nucleus (MVN) and the lateral vestibular nucleus (LVN). The MVN is divided into two subnuclei, the anterior and the posterior, which are thought to have different functions. The anterior MVN is involved in the regulation of eye movements and the posterior MVN is involved in the regulation of head movements.

The MVN receives input from the semicircular canals and the otolith organs and sends projections to the spinal cord and the brainstem. The LVN receives input from the vestibular nerve and sends projections to the brainstem and the spinal cord.

The MVN and LVN are both involved in the regulation of head movements during the performance of vestibular reflexes. The MVN is involved in the regulation of rapid eye movements and the LVN is involved in the regulation of slow head movements.


The location and the approximate numbers of the neurons which comprise the vestibular afferent system (VES) have been described for several mammals. The VES consists of approximately 100-400 neurons which can be identified by retrograde transport of HRP injected into the labyrinth. Vestibular afferent neurons (VEs) are cholinergic, have somatic diameters of 6-9 μm and are found lateral to the abducens nucleus, ventral to the medial vestibular nucleus, and under the medial aspect of the facial nerve.

We have used an antibody to choline acetyltransferase (Chat) and retrogradely transported HRP as markers for VEs and we have studied these neurons in the developing vestibular system of the rabbit (6-35 days). In neonatal rabbits VEs comprise a well defined nucleus. There was good qualitative agreement (less than 10% difference) in counting cells filled with HRP and stained for Chat. We have also stained VEs in VNS and Chat in 6-14 day old rabbits (N=5). However, in 21-35 day old rabbits only 50-200 VEs could be identified with the same methods (N=6). Previous experiments have shown that the vestibular afferent projection is bilateral with approximately 50% of the projection terminating in each hemisphere. However, in individual rabbits the ratio of contrateriaterally to ipsilaterally projecting VEs can vary by a factor of four. The decrease in the number of VEs projecting to the ipsilateral hemisphere and the variability in the ipsilateral/contrateral projection pattern may reflect important cellular events linked to the innervation of the peripheral vestibular apparatus. Because the death of VEs may also reflect one of the cellular mechanisms by which the vestibular primary afferent system is "calibrated" during development, and therefore might be influenced by neuronal activity in the peripheral or central vestibular system.

401.4 THE RESPONSE OF VESTIBULAR NUCLEUS NEURONES TO SENSORY CONFLICT. J.R Harris and J.W. Stellung. Department of Physiology, University of Wales, Cardiff, UK.

The vestibular nucleus (VN) is a site of convergence of canal, otolithic and visual signals. The timing constant of the VN is 10 ms, which suggests that this nucleus may filter sensory input. In addition, the VN is sensitive to changes in the vestibulo-ocular reflex (VOR) and is involved in the regulation of eye movements.

There are two types of VOR: the slow and the fast VOR. The slow VOR is a reflex that occurs in response to a change in head position and is mediated by the vestibular nuclei. The fast VOR is a reflex that occurs in response to a change in eye position and is mediated by the cerebellum.

VN neurons respond to changes in eye position, head position, and otolithic signals. They are also involved in the regulation of eye movements during different types of sensory conflict.

401.5 SODIUM, CALCIUM, AND POTASSIUM ION INFLUENCES UPON TRANSMISSION AT THE HAIR CELL-AFFERENT FIBER SYNAPSE OF THE FROG. S.L Cochran, Dept. Life Sciences, Indiana State Univ., Terre Haute, IN 47809.

This study is directed towards understanding how ions are involved in transmission between hair cells and afferent fibers in the isolated vestibular labyrinth of the frog. Ringer pipettes, intracellular recordings from lagena and canal afferents are digitized at 50 KHz for several minutes. EPS's (several thousand per cell) are detected by computer and cell spikes are integrated and their frequencies and amplitudes are quantified. Increasing the Na concentration of the solution bathing the hair cell led to a decrease in the mean amplitude of the EPS's (19 cells), suggesting that the hair cell transmitter opens a Na channel. Reducing the Ca concentration from 2 to 0.125 mM results in an increase in the mean amplitude of the EPS's (17 cells), suggesting that the hair cell transmitter opens a Na channel. Increasing the K concentration to 5 mM results in a decrease in the EPS frequency with no change in amplitude. The decrease in the K concentration to 0 mM decreases the firing rate of the hair cells, confirming the role of the hair cell release process in affecting the frequency in the membrane potential. These findings suggest that the subcellular channel may resemble Na channels, or the channel and indicate that this neuropil is very sensitive to small changes in ionic concentrations.

Supported by NSF (BNS 86-36736) and NASA (NAG 2-498).

401.6 NUCLEUS OF THE OPTIC TRACT (NOT); EFFECTS OF MUSCIMOL ON GENERATION OF OPTOKINETIC NYSTAGMUS (OKN), AND SUPPRESSION AND HABITUATION OF VESTIBULAR NYSTAGMUS. H. Bekinsch, B. Coho. Dept. of Neuro., Mt. Sinai Sch. of Med., NY 10029.

Single unit studies indicate that NOT processes visual motion signals that can produce ipsilateral slow phases of nystagmus (Hoffman et al., 1986; Mustard & Fuchs, 1989). Stimulation and lesions studies further indicate that NOT activates the velocity storage mechanism in the vestibular system to produce the slow component of horizontal OKN and OKAN (Kato et al., 1988; Schilt et al., 1988, 1990). In this study we injected the QAAs agonist, muscimol, into NOT of monkeys. They developed spontaneous nystagmus, with a slow component that was lost OKN and OKAN with ipsilateral slow phases. Per- and post-rotatory nystagmus and OKAN with contralateral slow phases were substantially prolonged, and all previous studies with contralateral slow phases were lost. Slow phases to the contralateral side, whether induced during OKN or per- or post-rotatory nystagmus, could not be suppressed by exposure to a stationary visual field. Habituation of vestibular nystagmus with ipsilateral slow phases was unaffected. These findings suggests that NOT participates in the control of slow phases of nystagmus, and that NOT processes visual motion signals that can produce ipsilateral slow phases of nystagmus.
401.7 COMPENSATION FOR HEMILABYRINTHECTOMY IN A RECURRENT NEURAL NETWORK MODEL OF THE VESTIBULO-OCCULAR REFLEX (VOR). I.J. Anastasio. Dept. of Otolaryngology, University of Southern California, Los Angeles, CA 90033.

Velocity storage is permanently lost following compensation for hemilabyrinthectomy. To gain insight into the possible mechanism of this loss, compensation was simulated as learning in a recurrent neural network model of the VOR. The three network layers represented horizontal canal afferents, vestibular nucleus neurons, and eye muscle motoneurons. Non-vestibular inputs were included. The network produced velocity storage via inhibitory commissural loops (CLs) between some VNNs.

Removal of the left canal input decreased VOR gain and unbalanced the network. Left VNNs inside CLs were driven into cut-off by the combined loss of canal excitation and increased commissural inhibition. This broke the CLs and eliminated velocity storage. Compensation was due primarily to increases in non-vestibular inputs. VNNs outside CLs regained their spontaneous rates, restoring balance and then VOR gain. Loss of velocity storage resulted from failure of VNNs inside CLs to also regain their spontaneous rates.


It has recently been shown that a direct pathway links the tuberomammillary histaminergic neurones with the vestibular complex. Since we have described, in vitro, two main neuronal cell types in the medial vestibular nuclei (which could correspond to vivo in the tonic and phasic neurones) we have tested their sensitivity to various agonists and antagonists of the histaminergic receptors.

Using intracellular recordings in slices we found that the bath-application of histamine induced in both neuronal subtypes a reversible increase in firing frequency. This effect was accompanied by a small membrane depolarization with no (or only minor decrease) change in membrane input resistance. In both cell types, the effect was mimicked by a histamine-agonist. When tested, Cimetidine (an H2 antagonist) was able to block the excitatory effect of histamine. Parallel studies performed in vivo with IP and local perfusion of the vestibular nuclei confirmed the functional implications of histaminergic receptors in the vestibular control of gaze and posture. Given that histaminergic drugs are widely used in the treatment of various vestibular syndromes including motion and space sickness, this model could be useful to investigate new therapeutics.

(Supported by a Swiss NSF grant no. 31-26495.89 and the french Ministere des Affaires Etrangeres).

401.9 ACTION OF THE OCCUPATIONAL HAZARDS ON THE VESTIBULAR SYSTEM. K.E. Trinu, ECA Foundation, 27 Lithuansky Str., apt.97, Kiev-71, Ukrain,

GSP 22500.

A total number of 544 persons were studied, 29 of them were control healthy group, 277 - stuff of the electric power station, 77 -tractor drivers, 95-velders, for examination Uemura's, Fukudas', pointing tests and oculography were used. Vestibular evoked potentials were recorded in the control group and in tractor-drivers. 93% of drivers complained vertigo. In this group were also found the disturbances in the performance of all the traditional clinical tests. On the oculograms were seen the low frequency components, which increased after the lateral gaze manoeuvres. The vestibular evoked potentials in this group had significantly increased latencies of the peaks. The welders had no signal of the vestibular damage. The oculogram had typical low frequency components, disappearing after the lateral gaze manoeuvres. Out of the electric power station stuff only 6.6% had vestibular impairment, 31.5% had mildly expressed changes of the vestibular function. The most important changes we seen due to the Uemura's and Fukudas' tests.

402.1 CIS-ACTING REGULATORY ELEMENTS FOR MBP TRANSCRIPTION IN MYELINATING CELLS. L.G. Wrafter*, S. Shuman*, H. Vogelbacher*, J. Grimmel, D. Plesner, and J. Kamholz, Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Characterizing the regulatory network of cis- and trans-acting factors necessary to effect the coordinate program of myelin-specific gene expression is central to understanding the regulation of myelin synthesis and assembly during development and remyelination. In order to identify the functional cis-acting elements necessary for tissue-specific expression of the myelin basic protein (MBP) gene expressed in both oligodendrocytes (OL) and Schwann cells (SC), we transfected MBP promoter-CAT (chimeric promoter acetyl transferase) fusion constructs into OL and SC. We have cloned the 150 base pair region upstream of the MBP gene contains sequences which are sufficient to promote CAT expression in a tissue-specific and orientation-dependent manner in both cell types.

We have extended this analysis and have found that the start site of MBP transcription, as determined by primer extension and RNAase protection studies, is identical in OL and SC, suggesting that the MBP promoter region should be the same in both cell types. We have also found that the cap site of the MBP promoter-CAT fusion transcript is identical to that of the endogenous MBP message.

Transfection of SC with a series of MBP promoter-fusion constructs, containing overlapping deletions within the 150 bp region sufficient for tissue specific CAT expression, demonstrated that the 5' boundary of a tissue-specific cis-acting element lies between nucleotides -190 and -150. Similar transfection studies in order to identify the 5' boundary of the tissue-specific element(s) used in OL.

402.2 DIFFERENTIAL PROLIFERATIVE RESPONSE OF ADULT HUMAN AND NEONATAL MOUSE ASTROCYTES TO CYTOKINES. R. Moundrian*, V.H. Yong and J.P. Antel. Neuroimmunology Unit, MNI, McGill University, Montreal, Quebec H3A 2B4, Canada.

In response to most forms of injury to the adult CNS, astrocytes undergo hypertrophy and/or hyperplasia. We have targeted cytokines as possible proliferative factors for astrocytes because infiltration of mononuclear cells is commonly seen after many types of CNS injury. Using a dual immunofluorescence (WAPF) technique (Yong and Kim, J. Neurosci. Methods, 21:9, 1987), we have identified the following as mitogens for cultured human adult astrocytes: supernatants from activated CD8+ or CD4+ human lymphocytes (proliferation index of 22.2 ± 2.3 and 16.5 ± 2.5), γ-interferon (6.5 ± 1.1), and interleukin-1 (6.2 ± 0.7). In agreement with these in-vitro results, γ-interferon when applied in vivo increased the extent of reactive gliosis in adult injured mouse brain. When cultured neonatal mouse astrocytes were treated, there was either no alteration (for interleukin-1), or a decrease in proliferation was observed (activated CD8+ supernatant; 40% of controls; γ-interferon: 60% of controls). We suggest that the intrinsic proliferative capability of astrocytes (low for adult human and high for neonatal mouse) determines the mitotic response to cytokines: proliferation for adult astrocytes, and differentiation/maturation with subsequent decrease in proliferation for neonatal astrocytes.
402.3 INVOLVEMENT OF PROTEIN KINASE C IN PROLIFERATION OF ASTROCYTES. W. M. Yong, Neuroimmunology Unit, MNI, McGill University, Montreal, Quebec H3A 2B4, Canada.

The objective was to assess the mechanisms by which proliferative agents for astrocytes exert their response. As determined using a GFAP-BrdU technique, we have identified the following cytokines as mitogens for cultured human adult astrocytes: supernatants from activated C8B-human lymphoma, -interferon and interleukin-1. For neonatal mouse astrocytes, rather than the above cytokines, fibrinolytic growth factor (FGF, 10 ng/ml) increased proliferation (250% of controls). To assess the mechanism of proliferation, we targeted the enzyme protein kinase C (PKC). Because of the PKC's selective (phorbol esters) produced proliferation of both human adult and mouse neonatal astrocytes. For human adult astrocytes, h-7 (a inhibitor of the PKC) strongly reduced the response of all mitogenic cytokines; 10 mM H-7 reduced proliferation by 50%, while 100 mM H-7 completely inhibited mitosis. For neonatal mouse astrocytes, h-7 in a dose-dependent manner reduced the basal proliferation, and also inhibited the mitogenic effect of FGF, ATP and dibutyryl cyclic AMP (PKA system) and the calcium ionophore A23187 did not significantly alter the proliferation of astrocytes. Thus, the enzyme PKC appears to be a common signal transduction pathway for agents identified to be mitogens for human adult and mouse neonatal astrocytes.

402.4 CELL CYCLE-SPECIFIC REQUIREMENT FOR MEVALONATE IN ASTROCYTES FROM LONG-TERM PRIMARY CULTURES Thomas J. Langan and Mary Slater*, Dept. of Neurology, S.U.N.Y. at Buffalo School of Medicine, Buffalo N.Y. 14222.

Astrocytes retain the capacity to divide after completion of brain maturation. We hypothesized that astrocytes in primary cultures can be stimulated to undergo the cell division cycle after prolonged quiescence, and that mevalonate, the precursor of isoprenoid lipids, regulates this process. DNA synthesis (uptake of [3H]thymidine into acid-precipitated material) declined by 85% with confluen ce in cultures of newborn rat brains in 10% calf serum (CS). After 2, 6, and 16 weeks the cultures were trypsinized and replated at 104 cells/cm2. 72 hours later, 79% of cells were astrocytes by glial fibrillary acidic protein immunofluorescence. Serum shift-down (10% to 0.1%) for 48 hrs followed by re-exposure to 10% CS resulted in 12% of quiescence (G0/M), then in a 6-fold increase (26/1534 vs. 4356 CPM/ug prot.) in DNA synthesis (5 phases). This increase was abolished by addition of mevalonate, a competitive inhibitor of mevalonate synthesis, and mevalonate reversed this block of DNA synthesis when added as late as 12h after serum stimulation. Cholesterol-rich lipoproteins failed to reverse the inhibition of DNA synthesis by mevalonate.

Thus, astrocytes after several months in primary cultures: 1. can be stimulated to re-enter the cell cycle; 2. require mevalonate or a mevalonate derivative thereof in late G1.

402.5 ALTERATIONS IN Na+ CURRENT CHARACTERISTICS DURING DEVELOPMENT OF HIPPOCAMPAL ASTROCYTES IN VITRO.


Na+ current expression in hippocampal astrocytes was studied during the first 20 days in vitro (DIV) using a single channel patch-clamp. The percentage of astrocytes expressing Na+-currents decreased from 75% at 1 DIV to about 30% at 10-20 DIV. Na+-currents could be differentiated into two types: a current with fast activation and inactivation, and a current expressed from a midpoint around -65mV and a second type with slower kinetics and an half midpoint around -85mV. The first type of Na+-current was seen in 1.5 DIV, the second type after 5-6 DIV. Current-voltage curves of Na+-current activation were identical in astrocytes of all ages. Na+-current densities displayed a biphasic time-course with an initial fast decrease during the first 5 DIV, followed by reexpression. At DIV 10 the Na-currents reversed at the same voltage as the Na+-currents of the first (midpoint -57 mV) to the second type of Na+-current (65mV), the latter only expressed later than 5 DIV. Interestingly Na+-current expression was restricted exclusively to astrocytes that were dye-coupled as assayed by following the spread of Lucifer Yellow. Transiently uncoupling cells with indocyanine did not mask Na+-currents. These results demonstrate that two types of Na+-currents are expressed in hippocampal astrocytes in vitro, and suggest a relationship between coupling and Na+-channel expression.

402.6 ACTIVATION OF ENDOTHELIN RECEPTORS REGULATES INTRACELLULAR Ca2+ IN CULTURED CEREBELLAR ASTROCYTES. A.A. Holmes, S.R. Glum and R.J. Miller, The Dept. of Pharm. and Physiol. Sciences, The University of Chicago, 547 E 58th Street, Chicago, IL 60637.

Sarothrombin (STX) is a selective endothelin antagonist that blocks in the adrenal medulla on the vascular smooth muscle endothelium. STX is known to be an agent that inhibits Ca2+ influx into cells. Infusion of a Ca2+ free medium (with 20 mM EDTA) caused a sustained increase in intracellular Ca2+ concentration lasting for 10-15 min. STX was also found to increase Ca2+ influx into cells, but STX was not able to induce or alter Ca2+ influx into cells. However, the effect of STX was abolished by the addition of indomethacin (10-8 M). The response to STX desensitized at high concentrations (100 nM), but did not do so at low concentrations (10 nM). Following the washout of STX, the [Ca2+]i plateaus declined rapidly in some cells but in other cells persisted for many minutes. In those cases, removal of extracellular Ca2+ caused a [Ca2+]i decline but the plateau was formed again on readdition of extracellular Ca2+. Thus, STX in addition to mobilization of Ca2+, appears to activate a Ca2+ permeable channel, that may be similar to that described in other cells following elevation of IP3.

402.7 CALCIUM WAVES PROPAGATE THROUGH ASTROCYTE NETWORKS IN DEVELOPTING HIPPOCAMPAL BRAIN SLICES. John W. Davis, Alex Chernyshyev* & Stephen J. Smith*, Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA 94305.

Using a laser confocal microscope and the calcium indicator dye fluo-3, we have detected waves of elevated intracellular Ca+ concentration that propagate through slices of neonatal rat hippocampus. At 20 degrees C, these waves propagate over distances of 1 mm or more at velocities of 10-20 micrometers per second. Because these Ca waves closely resemble those recently described in the rat hippocampus by Stergiou and others (Nature 361: 470-3, 1993), we suggest that the waves observed in hippocampal slices also propagate through coupled networks of astrocytes. Ca waves can be observed in the densest astrocyte layers, but are not observed in neocortex. We propose that these waves are not due to a selective inhibitor of Ca channels. The Ca waves may serve to propagate waves of Ca signals throughout the brain. Supported by the G. Harold and Leila Y. Mathers Charitable Foundation.


The propagation mechanism of Liddel's spreading depres sion (SD), which plays a role in the pathophysiology of migraines, remains enigmatic. Through a program of theoretical research I have now identified the probable mechanism. (1) A detailed analysis of the literature reveals that the fundamental propagation mechanism of SD is probably located in the glial compartment and has the nature of an extraordinarily slow (1) renewal of glial action potential, which helps trigger the neuronal depolarization of SD and is in turn facilitated by neuronally-released agents. (2) I have identified a widespread class of intracellular waves of excitation apparently based upon the Ca2+-induced release of Ca2+ from intracellular stores combined with cytologic diffusion of Ca2+ (cytocal wave). The propagation velocity of SD is typical of a cytocytic wave. The Arhenius activation energy of the conduction velocity rate-limiting mechanism of SD is typical of a cytocytic wave. The tendency of SD to occur under stressful or injurious conditions is common among cytocytic waves. The effect of an imposed constant electric field on the propagation velocity of SD is the effect expected on a cytocytic wave. On the basis of these and other arguments I propose with a high degree of confidence that a neuoglial cytocytic wave is the conduction velocity-determining mechanism of SD.

The three-dimensional morphology of mammalian oligodendrocytes is not well defined; these cells are not reliably stained by Golgi impregnations and they are difficult to reconstruct from EM images because of the tight connections between parent oligodendrocytes and their myelin segments are difficult to follow in serial sections. In a preliminary report (A. Bult and B. Ransom, Glia 2:470-475, 1989) we described the morphology of cells presumed to be oligodendrocytes in the intact optic nerve (RGN) using intracellular injections of horseradish peroxidase (HRP); these cells were characterized by the presence of 200-300 nm long, branched processes, each oriented along the long axis of the RGN parallel to the axons. The longitudinal processes of the cells were filled with HRP, which was transported to the cell body by thin branches, 15-30 nm long. By EM examination of such HRP-filled cells within the RGN, we have now directly determined their identity. Glial cells in 12-30 nm diameter optic nerve were filled with HRP and reacted using standard techniques. Three nerves containing isolated HRP injected cells with the morphology described above were examined, solely for viewing with transmission EM. HRP was found in paranoid loops at nodes of Ranvier and in the inner and outer processes of myelin sheaths, as well as in cell bodies. This distribution of HRP clearly identified these cells as myelin-producing oligodendrocytes. The longitudinal processes of these cells, as seen with light microscopy, correspond to the HRP-filled optic processes and therefore define the number (i.e., 10-20) and length (i.e., 200-300 μm) of the internodal myelin sheaths provided by individual oligodendrocytes. Since the entire cytoplasmic border surrounding the myelin sheath readily filled with HRP, this compartment could serve as an efficient conduit for metabolites or membrane constituents. Supported by NIH grant NS 15589.


Intracellular pH (pHi) was measured microfluorometrically in single astrocytes cultured from neonatal rat brain, using the pH-sensitive dye BCECF. In many cell types, brief exposure to NH4Cl/NH4+ causes an abrupt acidification, recovery from which can be used as an assay for mechanisms responsible for pHi regulation. In astrocytes, we now report that application of NH4+ (1-2 mM) causes a tetrapodal change in pHi. For example, in five cells 5 mM NH4Cl/NH4+ causes: (1) a recovery from 6.90 ± 0.03 to 7.23 ± 0.03, presumably due to NH4+ entry; (2) a subsequent slow decline to 7.03 ± 0.02, perhaps due to NH4+ entry; (3) a slower increase to 7.29 ± 0.05; and finally (4) a subsequent decline to a new stable level of 6.88 ± 0.04. The subsequent removal of NH4+ causes an abrupt acidification from which the pHi rapidly recovers. However, the rate of recovery at any pH i depends uniquely on that pH i, but not on the history of the experiment (e.g., the recovery is slower when the pH i goes to very acidic levels following NH4+ removal). In contrast, brief removal of external Na+ (5 min) causes a monophasic fall in pH i from 6.88 ± 0.02 to 6.36 ± 0.02, with little pH i recovery. Removal of NaCl leads to a rapid pH i recovery. The recovery rate at any pH i depends only on that pH i, but not on the history of the initial pH i or the decline to which the pH i fell during the period of Na+ removal. This recovery rate is inhibited 40% by brief removal of external Cl-; <5% by pretreatment with 50 μM DIDS, and ~75% by Cl- removal and DIDS-preface. The Na+ and Cl- and DIDS-sensitivity of the recovery suggests that a Na+-dependent Cl-HCO3 exchanger can be operational in the nominal absence of HCO3, or that Cl- removal or DIDS could affect a Na+-dependent, HCO3-independent transport process.

The cytokine IL-1, a major participant in immune and inflammatory responses, is present in the CNS and specific receptors appear to exist in brain. To assess a possible interaction between IL-1 and the GABAergic system, we evaluated effects of IL-1 on GABA-dependent chloride uptake in mouse cortical synaptoneurosomes. Uptake was determined using the GABA analog muscimol over a 6 sec interval. Preincubation of synaptoneurosomes with IL-1, 100 pg/10 ng/ml, augmented maximal chloride uptake by approximately 40%. There was no significant alteration in the EC50 for muscimol with IL-1. At both higher and lower concentrations of IL-1, there were no significant effects on GABA-dependent chloride uptake. Addition of IL-1 concurrently with muscimol had a smaller, but still significant increase in chloride uptake at IL-1 concentrations of 100 pg/ml and 1 ng/ml. A specific IL-1 receptor antagonist, 10 ng/ml, prevented the effect of 100 pg/ml or 1 ng/ml IL-1 on maximal chloride uptake. These data indicate that IL-1, acting at a specific binding site in brain, augments GABA-dependent chloride uptake in cortical membrane preparations.

INTERLEUKIN-1 INDUCES c-FOS AND c-JUN mRNA EXPRESSION IN AT-T20 CELLS BY A MECHANISM INDEPENDENT OF PROTEIN KINASE C AND PROTEIN KINASE A. Mirela O.Fagarasan*, Katherine Muegger,* Francesca Airolo*, S.K. Durum* and J. Axelrod* (SPON: J.Axelrod) (NIDDK, Div. of Endocrinology, National Institute of Mental Health, Bethesda, MD) and *National Cancer Institute, Frederick, MD

We have demonstrated that IL-1 after a long period of treatment directly stimulates 3H-endorphin release and potentiates the effects of other secretagogues in AT-T20 cells, a mouse anterior pituitary cell line (Fagarasan et al., PNAS, 86, 2070-2073 ). It was also observed that IL-1 markedly phosphorylated 19-, 20- and 60 kDa proteins (Fagarasan et al., PNAS, 87, 2556-2559). We found that another early signal triggered by IL-1 in AT-T20 cells involves the enhancement of c-fos and c-jun mRNA expression. The effect appeared within 30 minutes, is transient and returned to basal levels after 2hr. Desensitization of protein kinase C by phorbol ester (TPA) pretreatment had no effect on the ability of IL-1 to induce c-fos and c-jun mRNA expression. An experiment was designed to investigate whether IL-1 can still generate this early signal after its continuous presence with AT-T20 cells for 24 hours. After prolonged treatment with IL-1, the capacity of IL-1 to induce c-fos mRNA expression was abolished, but TPA and CRF effect on c-fos mRNA was markedly expressed. Whether the interaction of these intermediate early gene products is involved in IL-1 potentiation of 3H-endorphin secretion induced by secretagogues awaits further investigation.

CALCITONIN GENE RELATED PEPTIDE (CGRP) AND ITS RECEPTORS IN THE MOUSE THYMUS AND SPLEEN. K. Bulloch, J. Hausman*, T. Meinelshchuk, T. Radolicic*, S. Sharp*, D.H. Simons, L.M. Swanson*, Dept. of Psychiatry, Univ. of Cal. San Diego, La Jolla, CA 92039; Salk Institute, San Diego, CA 92121

In this study we demonstrate with immunocytochemistry the presence of CGRP in intrathyphic mast cells, thymus and a discrete population of cells at the corticomedullar boundaries. In situ hybridization confirms that the subcapsular and trabecular mast cells and the intrathyphic cells at the corticomedullar boundary synthesizes messenger RNA for CGRP. Little to no CGRP activity was observed in the nerves or cells of the spleen. However, receptor sites for CGRP can be detected in both spleen and thymus tissue. These sites bind iodinated CGRP with a KD of 14nM (Bmax 8 fmoI/mg protein) for spleen and 12nM (Bmax of 18 fmoI/mg protein) for thymus. These data indicate that CGRP may play a role in lymphocytic immune interactions. Supported by ONR grant #N00014-89-J-1256.


Acute administration of endotoxin (lipopolysaccharide, LPS) as well as interleukin-1 (IL-1) stimulate hypothalamic norepinephrine turnover by a prostaglandin dependent mechanism. The rapid adaptation to the physiological effects of endotoxin is well known as endotoxin tolerance. The neurochemical consequences of chronic endotoxin administration have not been studied. We administered LPS or saline to male C57BL mice (5 ug/mouse/day, ip) for 7 days. On day 8, animals were challenged with saline, LPS (5 ug/mouse), IL-1 (1 ug/mouse) and tumor necrosis factor (TNF, 1 ug/mouse) and decapitated after two hours. Blood was collected for corticosterone and brain parts dissected for determination of catecholamines, serotonin and metabolites. The stimulation of hypothalamic norepinephrine turnover observed after acute LPS administration was absent following chronic LPS administration. However, the acute LPS stimulated elevation of corticosterone were unaffected by chronic administration of LPS. These results suggest that endotoxin desensitization occurs at the level of the macrophage in response to endotoxin. Other neurotranscendine, cytokine-mediated responses appear to be unaffected following desensitization of the macrophage response to endotoxin.

SUPPRESSION OF NATURAL KILLER CELL ACTIVITY FOLLOWING ELECTRICAL STIMULATION OF THE RAT MESENCEPHALON. R.J. Weber and A. Pert. Neuroimmunology Unit, Laboratory of Medicinal Chemistry, NIDDK, NIH, and Biological Psychiatry Branch, NIMH, Bethesda, MD 20892

We have previously reported that microinjections of morphine into the periaqueductal gray matter (PAG) of the mesencephalon produce suppression of natural killer cell (NK) activity (Weber and Pert, Science : 245: 188, 1989). Electrical stimulation of the periventricular region and the PAG have also been shown to produce opioid-like effects in the rat. The purpose of this paper was to determine whether electrical stimulation of the PAG would also suppress NK cell activity. Rats were implanted with bipolar electrodes aimed for various rostral and caudal regions of the PAG. One week following recovery the animals were stimulated with intermittent 150 msc trains of biphasic pulses (50usec in duration presented at 100Hz and 0.005uA) for 20 minutes. Spontaneous NK activity was determined 3 hours following termination of stimulation. The most active sites in suppressing NK cell activity were found in the caudal PAG, just lateral to the dorsal raphe. Injections of morphine into a similar region were also found to suppress NK cell activity in the previous study cited above. Additional sites that were involved in alteration of NK cell activity were found in the caudal periventricular gray matter extending from the level of the posterior hypothalamus to the red nucleus. Studies are underway to determine the neural circuitry through which these signals gain access to the immune system.

REGULATION OF PRE-PROENKEPHALIN A (PPEA) GENE EXPRESSION IN NORMAL MURINE T LYMPHOCYTES. K.M. Linner*, S. Nicol and B.M. Sharp*. Minneapolis Medical Research Foundation and Departments of Medicine, Hennepin County Medical Center and University of Minnesota, Minneapolis, MN 55404

Recent studies have shown that cells of the immune system can express genes for endogenous opioids. For example, PPEA mRNA has been found in transformed T cell lines, primary and normal murine macrophages and mast cells, and concanavalin A (Con-A) stimulated murine T helper cell clones. Our studies extend these findings regarding PPEA gene expression and signal transduction. Con-A and Thymocytes and splenocytes isolated from female CD-1, SPF mice (4-5 w.o.) were cultured with several doses of Con-A for 24, 48, and 72 hr. Poly A mRNA was isolated and either dot or Northern analysis was done. PPEA mRNA was identified by hybridization with a specific 50-mer ZP-CDNA. Only thymocytes were induced to express PPEA mRNA when cultured with Con-A. These cells responded to 15 ng/ml Con-A (baseline) occurring after 72 hr in culture at Con-A doses of 5-7.5 ng/ml. No PPEA mRNA was detected in thymocytes after 24 hr in culture, whereas low levels were evident at 48 hr. Two hybridizable bands were found in cultured cells, one corresponding in MW to putative PPEA (14000) and one larger. No PPEA mRNA was detected in splenocytes at any time in response to any dose of Con-A, even though these cells, like thymocytes, were induced to proliferate by Con-A. PPEA mRNA expression was further shown to be inducible in CD4+ thymocytes and its expression was inhibited by IL-1-beta. A role for enkephalin peptides in the ontogeny of T cells in the thymus is postulated. (Supported by DA04196)
403.9

CRH stimulates rat B cell proliferation in vitro at nM concentrations (McGillis, et al., J. Neurosci. Res. 31:166, 1989) and CRH binding sites characterized as specific for CRH in peripheral tissues. CRH binding sites on B cells were characterized on human IM-9 cells. Binding studies were done with intact cells using 125I-(Nle3,8)-hCRH binding. Specific binding, specific and nonspecific binding, and time and temperature dependent. Specific binding had a linear dependence on cell concentration at cell densities ranging from 2.5 to 20 x 10^5 cells/ml. Equilibrium binding was dependent on temperature, with equilibrium reached at 3 hr at 27° C and by 90 min at 37° C. No binding was observed at 4° C. Best fit analysis of saturation isotherm binding suggested a two site model, with a high affinity KD of 17.8 ± 2.15 pM and a low affinity KD of 2.71 ± 0.78 nM. Binding site densities were 181 ± 97 sites/Cell and 4363 ± 2094 sites/cell, respectively. The presence of CRH binding sites on human B lymphoblast supports an immunomodulatory role for CRH in peripheral tissue.

403.11
INTIMATE CONTACT BETWEEN RAT BASOPHILIC LEUKEMIA (RBL) AND MELANOCYTOMA (PC-12) CELLS COMBINED IN CULTURE. L. Solomonides*, V. Dimitriades, M. Holdridge* and T.C. Sheehan*. Dept. of Pharmacology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111

Mast cells are important in allergic reactions. However, they have recently been implicated in a number of neuro-endocrine disorders and have also shown to be in close contact with neurons. Here, we investigated the possibility that interactions analogous to mast cells and cells may exist in culture. In vitro, RBL cells, which are homologous to bone marrow-derived mast cells, and PC-12 cells, which are derived from the pheochromocytoma, were grown in culture at a 1:1 ratio of about 3-5x10^5 cells and were examined after 5 days. The cells were grown in culture dishes with sterile cover slips which were fixed for light, as well as scanning and transmission electron microscopy. Close contact was evident directly between the cells and between PC-12 axonal processes and RBL cells. Histoamine measurements after section induced by the cation ionophore A23187 indicated an absence of the cultures containing both IL-3 and NOF. These results suggest that mast cells may be immunoregulated directly and suggest the possibility that such mast cells may also express different phenotypic and functional characteristics.

404.1
THE ROLE OF POSTSYNAPTIC ACTIVITY IN ESTABLISHING APPROPRIATE SYNAPTIC MORPHOLOGY DURING NEUROMUSCULAR DEVELOPMENT IN DROSOPHILA EMBRYOS. M.D.S. Anderson and H. Keshishian. Dept. of Biol, Yale New Haven, CT 06511

Through the use of immunocytochemistry and AGTX, a toxin isolated from the venom of the Orb Weaver spider Araneus gemma that blocks glutamate activity post-synaptically at the neuromuscular junction in Drosophila, we have shown that the early perisynaptic movements in Drosophila embryos are mediated by glutamate and weakly neurotoxic. Early growth cone contacts on the bodywall muscles in stage 16 embryos are immunoreactive for glutamate and coordinated perisynaptic bodywall muscle movements begin to be visible during this stage of development. All of the coordinated muscle contractions are abolished following the microinjection of AGTX. It is clear that neuromuscular activity is present during this stage and that the stereotyped ending analyses begin to be established on Drosophila bodywall muscles. Therefore, we sought to identify whether activity is necessary for the acquisition of a proper synaptic morphology. During stage 17 of embryogenesis the endings on the bodywall muscles begin to resemble the stereotypical mature larval endings. We have used AGTX to block glutamate activity in stage 17 embryos, followed by HRP immunocytochemistry to look at the role of postfunctional activity in the development of stereotypical ending patterns and synaptic boutons. There appears to be no effect on the establishment of normal ending morphology when the postneuronal response is blocked. We have also begun to microinject TTX into stage 17 embryos to determine whether blocking action potentials during this stage affects the development of appropriate synaptic morphology.

404.2

The expression of the cell surface chondroitin sulfate proteoglycan identified by monoclonal antibody Cat-301 on motor neurons is dependent upon normal activity during a circumscribed period postnatally. The NMDA antagonist MK-801 inhibits Cat-301 expression in a stereotactic, dose-dependent manner when administered to neonatal or adult hamsters. We have confirmed that this effect is mediated through the NMDA receptor using a second NMDA receptor antagonist, aminophosphononic acid (APV). APV was incorporated into the polymer Elvas and slices implanted over the lumbar enlargement. In neonates, APV specifically inhibits Cat-301 development on motor neurons in a dose-dependent manner, while in adults APV has no effect on Cat-301 expression. Two sets of experiments suggest that NMDA receptor antagonists are operating at the spinal segmental level. First, we compared the effects of APV implants placed over the lumbar enlargement to those placed over the cervico-medullary junction (CMJ) or the sensorimotor cortex (SMC). Implants at the lumbar enlargement were twice as potent as 301 implants in inhibiting Cat-301 expression, while SMC implants had no effect on Cat-301 expression. The decrease in efficacy of APV with increasing distance from the lumbar enlargement suggests that NMDA receptor antagonists exert their effects in this system at the spinal segmental level. Second, to test whether afferents from normally active muscles provide the coextraneous activity to motor neurons necessary for activation of the NMDA receptor, electrical activity at the spinal segmental level was blocked by implanting a TTX-coated wire into the sciatic nerve. Cat-301 expression was inhibited on motor neurons connected to TTX-alkaloid implants but not hindlimb muscles evoke patterned electrical activity at the segmental level, activating NMDA receptors and leading to normal maturation of motor neurons as assayed by Cat-301 expression.
404.3  PHENOTYPIC DIFFERENTIATION OF STRIATAL TRANSPLENED NEURONS IN RELATION TO DOPAMINERGIC HOST AFFERENTS. B. D'Amato, J. B. McLean, and E. Hanson. INSERM U161, 2 Rue d'Albe, 75018 Paris, France.

The role of dopaminergic (DA) afferents in the acquisition of a specific phenotype by their post-synaptic cells was investigated in embryonic suspensions transplanted into either a kainic acid-lesioned striatum (KA animals) or a KA striatum additionally deprived in DA by 6-OHDA administration (KA-6-OHDA animals). The expression of the Di-1-associated protein DARPP-32 was assayed immunohistochemically at 4 to 60 days following transplantation (T4 to T60) and paralleled with the development of DA afferents from the host. Acetylcholine esterase (AChE) histochemistry was performed on adjacent sections.

Striatal embryonic neurons already expressed DARPP-32 at the time of their dissection (E16). However, no immunoreactivity was observed within the transplant tissue until T6. This delay was prolonged until T14 in KA-6-OHDA animals.

As demonstrated by AChE histochemistry, transplants of both experimental groups progressively developed the specific patch pattern corresponding to the segregation of striatal and extra-striatal tissue. In addition, both the survival and the general morphology of transplanted cells were similar in KA and KA-6-OHDA animals.

Homotopically transplanted striatal neurons, if transiently impaired by their displacement, are able to rapidly recover their specific DARPP-32 phenotype. The role of DA afferents in the acquisition of this expression and in the general development of striatal cells is to be rather limited since their elimination, although introducing some supplementary delay, does not suppress the expression of DARPP-32.

404.5  REGULATION OF NEUROTRANSMITTER PHENOTYPE IN THE NEMATODE C. ELEGANS. C. M. McNew and C. J. Kenyon. Dept. of Biochemistry and Biophysics, Box 0054, Univ. of California, San Francisco, CA 94143.

Specification of a neurotransmitter phenotype requires, among other characteristics, the coordinate expression of appropriate biosynthetic enzymes. Synthesis of the biogenic amines dopamine (DA) and serotonin (5HT) require the expression of an aromatic amino acid (AAA) hydroxylase and decarboxylase. In the nematode C. elegans, SHT and DA are used by a number of identified neurons, including a intestine neuron required for egg laying and sensory neurons necessary for the mating. Genes that specify neuronal characteristics in C. elegans can be identified by mutation. As a first step towards analyzing the genetic control of neurotransmitter and other neuronal phenotypes, we are cloning genes encoding biosynthetic enzymes for the biogenic amine neurotransmitters to use as markers of specific neuronal differentiation. We used PCR with degenerate oligonucleotides encoding highly conserved regions of AAA hydroxylases to amplify a genomic fragment encoding a C. elegans AAA hydroxylase. Of 5 clones sequenced, 2 encoded an AAA hydroxylase; in the region sequenced, 27 of 36 amino acids were identical to human tyrosine hydroxylase. The sequence included a typical C. elegans intron at an evolutionarily conserved splice site; typical C. elegans codon usage was also apparent in the coding region sequenced. We have isolated a 3.5-kilobase clone that we are using to isolate cDNAs for sequencing. We probed a genomic southern blot and found that the gene is present as a single copy in the genome.

We plan to use in situ hybridization to determine sites of expression of the gene. We also plan to fuse β-gal into the AAA gene and use worms containing such a construct to identify mutations that alter its expression and to examine the expression in certain known mutants. Such a screen should yield genes that are required for the regulation of neurotransmitter synthesis as well as genes that determine which neurons produce a specific neurotransmitter.


NPY is the most abundant peptide in the nervous system. The fetal rat mesencephalon already contains substantial amounts of NPY, which increase dramatically around the time of birth. The aim of this study was to establish an in vitro model that permits the study of the mechanisms controlling the production of NPY in the perinatal brain. Single cell suspensions were prepared from the hypophyseal-pituitary-thyroid (HPT) axis of 15-day-old rat fetuses, aggregates were formed by cell-cell adhesions, and synaptic connections were established by Rz (NPY-IR). Aggregate NPY-IR doublet between 0.3 to 12 days of culture (1 to 3 mg/ml). At 12 days in vitro, the amount of NPY-IR in the medium exceeded those in the aggregates 5-fold. Size-fractionation of NPY-IR indicates the presence of both proto-NPY and NPY-IR and that their molar ratio is much higher in the aggregates. Interestingly, HT-112 neurons contained both proto-NPY and NPY-IR, but tissues of 9-day-old rats contained only NPY-IR, suggesting that NPY-IR is regulated during the biosynthesis and processing of proto-NPY. Thus, the cultured aggregates produce and secrete large amounts of NPY-IR and NPY-IR, and both production and secretion increased with culture age. Hence, the cell aggregates provide an excellent model for studying developmental regulation of proto-NPY biosynthesis/processing.

404.8  DIFFERENTIATION OF ADRENERGIC CELLS IN A CHOLINERGIC GANGLION. B. D. Heathcote, A. Chen, and M. Bennett. Department of Biological Sciences, University of Wisconsin, Box 413, Milwaukee, WI, 53201.

While studying the development of a parasympathetic ganglion, we discovered a discrete population of cholinergic neurons that co-exist with adrenergic neurons at the same time and in the same location as cholinergic neurons. The cardinal ganglion of the frog, Xenopus laevis, contains cells that had the characteristics (small size, catecholamine fluorescence and multiple granular cell bodies) of SIF (small intensely fluorescent) cells.

SIF cells were present in some ganglia before cholinergic neurons differentiated. They were also present at 2.1 days of development, when the first cholinergic precursors were becoming postmitotic. Although few SIF cells were in the heart at this stage, they were concentrated on the left side.

The development of these two cell types appeared to be coordinated, since SIF cells were maintained at a local level in all ganglia by postmitotic cholinergic neurons. SIF were clustered in the sinus venosus portion of the cardiac ganglion. At early development, the first cholinergic neurons were localized in the same area. Thus, within this small region, neural crest cells simultaneously adopted two different fates.


In the developing mesencephalon of the rat, the dopaminergic (DA) cells are generated in the ventricular zone of the basal plate between E11 and E15. The DA cells align and appear to move along radial glia as they migrate from the ventricular zone through the mantle layer to the ventral surface of the mesencephalic flexure. At about 16 days, NACAM immunoreactive (NACAM-IR) material was noted on the surface of cells in the ventricular zone and throughout the two layers. The distribution of NACAM-IR material was not uniform. Cells along the ventral surface of the mesencephalon, the area occupied by DA cells, had the greatest amount of NACAM-IR material. Cells in the ventricular zone contained a moderate amount of NACAM-IR material. Cells between these two regions contained a lower amount of NACAM-IR material. Our data suggest that the level of expression of NACAM on the surface of developing DA cells of the mesencephalon may influence migration and/or expression of TH.
404.9 EARLY EXPRESSION OF POTASSIUM CHANNEL TRANSCRIPTS IN THE AMPHIBIAN SPINAL CORD. A.B. Ribera, Dept. of Biol., UCSD, La Jolla, CA 92093 and Dept. of Physiol., Univ. of CO, Denver, CO 80262.

The delayed rectifier potassium current in amphibian spinal neurons exhibits a specific timetable of functional differentiation, that is coordinated with the maturation of calcium currents to permit developmentally regulated action potential phenotypes. Molecular probes for a Xenopus nucleotide sequence encoding a potassium channel have been identified by homology screening with the Drosophila Shaker sequence (Tempel et al., 1987). The Xenopus sequence is most related to the mammalian sequences RBK2 (McKinnon, 1989) and MK2 (Chandy et al., 1990) and is thus called XSh2. As reported for some mammalian sequences (Chandy et al., 1990; McKinnon et al., 1990), the coding region is contained within a single uninterrupted exon. Southern analysis of genomic DNA with XSh2a suggests that it is a member of a family of closely related genes. The predicted peptide has 493 amino acids.

Functional expression of XSh2a in oocytes induces a delayed rectifier potassium current. RNAse protection assays indicate that XSh2a is expressed in the nervous system but not detectable in the excitable tissues of heart or skeletal muscle. Its transcription is first observed in vitro at the neural tube stage and thus slightly after NCAM, an early marker of neural differentiation. In cultured embryonic amphibian neurons, the transcript is present at the time of initial morphological differentiation.

The timing of the functional expression and maturation of the delayed rectifier is fundamental for the characteristic maturation of electrical excitability in amphibian spinal neurons. The tissue-specific and temporal patterns of expression of XSh2a indicate that this program may be established at the level of gene transcription. Supported by NIH grants NS25217 (ABR) and NS25916 to Nicholas C. Spitzer.

404.11 DIFFERENTIATION OF DELAYED RECTIFIER POTASSIUM CURRENT IN EMBRYONIC AMPHIBIAN MYOCYTES. N.C. Spitzer & A.B. Ribera. Dept. of Biology & Center for Molec. Genetics, UCSD, La Jolla, CA, 92093.

The developmentally regulated expression of prolonged outward potassium currents influences the extent to which sustained inward currents contribute to the action potential at early stages of differentiation. In amphibian spinal neurons, the long duration and calcium-dependence of the embryonic action potential and the amount of calcium influx are largely determined by the extent of maturation of the delayed rectifier potassium current (Boris, 1986; O'Dowd et al., 1988; Spitzer, 1988).

We have undertaken a parallel study of differentiation of myocytes, in which action potentials are brief and sodium-dependent even at early stages. The early expression of electrical excitability in embryonic amphibian myocytes growing in culture has been examined previously using intracellular voltage recording techniques (DeCino and Kidokoro, 1985; Henderson and Spitzer, 1986). The membrane exhibits a delayed rectification in response to depolarization at times earlier than those at which impulses can first be generated.

We have examined the differentiation of this outward current in embryonic myocytes using whole cell voltage clamp. IK, is initially absent. When first recorded it is small and slowly activating but undergoes six-fold increases in both density and rate of activation during the first day in culture. This maturation is dependent upon transcription, and its rate and extent are influenced by the presence of other trans factors. The amplitude of the outward delayed rectifier is longer than the inward calcium current at all stages examined and prevents expression of long duration action potentials. Supported by NIH grants NS25217 (NCS) and NS25916 (ABR).


Immunocytochemical localization of ER is the only method that allows examination of the ultrastructural characteristics of estrogen receptor cells. We have examined ER neurons in the VL, as a role for these neurons in sexual behavior has substantial support. We have characterized animals were perfused with 4% paraformaldehyde. ER was localized in vibratome sections using NRZ, a rat monoclonal anti-ER antibody (Aberbalt Laboff), the Vector Elite kit, and silver intensification of DAB reaction product. Sections were post-fixed in O.OS and embedded in Epon. All ER neurons had large cytoplasmic processes in nuclear ratios; prominent proximal dendrites; a large Golgi apparatus; stacks of rough endoplasmic reticulum and secretory granules. These are characteristics of cells that make peptidoglycans. ER neurons showed varying degrees of immunoreactivity that could not be attributed simply to depth within the section. ER was concentrated in the nucleus with the nucleolus being free of deposits. Light reaction product was also seen in both dendritic and somal cytoplasm. The dendrites and cell bodies were heavily innervated with the most common presynaptic element being Gray's Type I terminals (a prominent synaptic cleft, little pre- or postsynaptic densities and accumulations of small, round, clear vesicles). The pronounced input to these cells strongly suggests that both synaptic and hormonally guided neuron activities. Supported by HD 10065 (AJS) and HD 22583 (LIH).


In a previous study, we demonstrated that progesterone (P4) and the synthetic glucocorticoid, triamcinolone acetinate (TA), but not cortisol (F), could induce LH and FSH release in estrogen-primed ovariectomized rats. Therefore, the purpose of this study was to determine if the stimulatory effects of P4 and TA on LH and FSH release were associated with changes in LHRR or NPY concentrations in the medial basal hypothalamus (MBH) or parietal area (POA). Ovariectomized estrogen-primed immature rats received either vehicle, P4, TA or F (1mg/kg BW) at 0900h on day 29. Animals were killed at 0900, 1200, 1500 and 1800h for serum LH and FSH measurements and the MBH and POA were dissected and analyzed for LHRR and NPY concentrations via RIA. P4 and TA-treated animals showed significantly elevated LHRR on days 1200 and 1500 and at 1800h. F was without effect. P4 significantly increased MBH LHRR and NPY concentrations at 1200h followed by a significant fall at 1300h. TA caused a significant increase in MBH LHRR or NPY at 1200h but, as with P4, there was a significant fall in MBH LHRR and NPY at 1300h. Consistent with its lack of effect on LH and FSH, F had no effect on MBH LHRR and NPY. With respect to the POA, P4-treated animals showed a significant fall in LHRR concentrations at 1300h as compared to 1200h. No effect was seen on POA. TA had no effect on POA LHRR or NPY. Interestingly, F significantly increased POA LHRR and NPY at 1300h followed by a fall at 1300h. However, this change in POA LHRR and NPY may not be related to gonadotropin secretion since F had no effect on LH and FSH levels. In summary, the stimulatory effects of P4 and TA on LH and FSH release appear to be mediated by a similar mechanism involving changes in MBH LHRR and NPY concentrations. Supported by HD16688, HD00727, March of Dimes 11118.
STEROIDS: RECEPTORS AND ACTIONS

STEROID REMOVAL INCREASES GnRH SECRETION IN THE EWE

505.3

STEROIDS: RECEPTORS AND ACTIONS

Stereoid Removal Increases GnRH Secretion in the Ewe

505.4

Progestosterone Enhances Fos Expression in LHrH Neurons During an LH Surge

Society for Neuroscience Abstracts, Volume 16, 1990

THURSDAY AM

505.5

Effect of Estradiol (E2) on Norepinephrine (NE)

Levels in the Proeoptic Area (POA) of Anestrous Ewes

505.6

Progestosterone in Vitro Rapidly Reduces

Nordrenergic agonist-stimulated cAMP Accumulation in Hypothalamic Slices

505.7

Dihydrotestosterone Inhibits Estrogen-induced Pituitary Hypertrophy and Hyperprolactinemia in Both Male and Female Rats

505.8

Ultrastructural Localization of Estrogen Receptor-immunoreactivity in Guinea Pig Hypothalamus

505.4

Progestosterone Enhances Fos Expression in LHrH Neurons During an LH Surge

W. S. Lee, M. S. Smith, G. E. Hoffman

Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261

The ability of progesterone (P) to enhance the LH surge is well established in the rat. However, whether its primary site of action is on the pituitary or ovarian levels is uncertain. Recently, we have demonstrated that LH surges induced by estradiol benzoate (EB) and EB plus P (Hoffman et al., J. Endocr. 117:590 and the events of proestrus (Lee et al., JRNS, 1990, in Press) are accompanied by induction of Fos in LHrH neurons in the proptic area and anterior hypothalamus, suggesting that stimulation of activity of the LHrH neurons is the primary drive for the LH surge. To determine the role of P on this process I intact female rats were treated with P antagonist RU 486 (5 mg at 120 h on proestrus and killed at specified times during the afternoon and evening for comparison with untreated proestrus rats. P-primed rats were treated with EB (5 µg) and then were treated with E alone (50 µg) or EB plus F (5 mg). The brains were processed for in situ hybridization with anti-Fos antibodies and for in vivo autoradiography of Fos and LHrH. RU 486 treatment dramatically reduced both the magnitude of the LH surge and the degree of Fos induction (numbers of cells expressing Fos and intensity of Fos staining) in LHrH neurons during proestrus. Also, the magnitude of the LH surge and degree of Fos expression in LHrH neurons induced by EB-P treatment were greater than that induced by EB treatment alone. These data suggest that a major site of P action is on hypothalamus and P enhances the Fos signal by greatly increasing LHrH neuronal activation. Supported by NIH grant HD 13254 and HD 14645

505.5

Effect of Estradiol (E2) on Norepinephrine (NE)

Levels in the Proeoptic Area (POA) of Anestrous Ewes


AFRC Inst. of Animal Physiology and Genetics Res, Babraham, UK

CRES AND PITT, West Virginia Univ, Morgantown WV 26506

Inhibitory Neuron input to the POA appears to play a role in the negative feedback action of E2 in anestrous ewes. This study examined the effects of E2 on NE levels in the POA of ovariectomized (OVX) anestrous ewes and on the response to the α-adrenergic antagonist, phentolamine (PBZ) using microdialysis probes (5 mm membrane length). Probes were inserted via chronic guide tubes into the POA of conscious ewes and either Ringer (R) solution (0.5, 12-20h) or 60 µg/ml PBZ in R (8-12h) pumped through the probes at 2 µl/h. Dialysate samples were collected every 20 min and concentrations of amine-containing transmitters and GABA measured using HPLC with electrochemical or fluorometric detectors. Dialysate was done with and without E2 (1 cm-long Silastic capsule sc for 2 days) in each of ten ewes. Mean NE levels during 2-8h of dialysis were lower in OVX-E vs E (477 ± 86 pg/ml) than in OVX (1277 ± 243 pg/ml; p < .05), but E increased the variability of NE (intra-animal CV increased from 16 ± 3% to 31 ± 2%, p < .01). E had no effect on cAMP, dopamine, serotonin, or GABA levels in the POA. Perfusion of PBZ through the probe increased NE levels, presumably by blocking adrenergic autoreceptors. However, this effect only occurred when probes were located rostral to the decussation of the anterior commissure (rostral probes: 424 ± 60% of controls; caudal probes: 111 ± 18% of controls: p < .01), suggesting that the autoregulatory control of NE release varies in different areas in the rostral hypothalamus. In conclusion, these data show that E increases NE levels in the POA of anestrous ewes, but may increase its episodic release. (HD 17864)

505.7

Dihydrotestosterone Inhibits Estrogen-induced Pituitary Hypertrophy and Hyperprolactinemia in Both Male and Female Rats

X. X. Guan*, A. J. Carrillo, and P. C. Dobhney

Dept. of Anatomy, Northeastern Ohio Univ. Coll. Med., Rootstown, OH 44272

Prolonged treatment with estrogen (E2) causes pituitary cell hypertrophy, hyperplasia, and hypersecretion of prolactin (PRL). We have previously shown that co-administration of dihydrotestosterone (DHT) can inhibit this effect in pituitaries under hypothalamic control, but not those transplanted to an ectopic site. Since the donors used in the transplant study were female rats, the present experiments were undertaken to remove (1) differences that exist between male and female pituitaries in their response to E plus DHT. Adult male and female Fischer 344 rats were castrated and treated with subcutaneous implants of crystalline testosterone (T), E, or E + DHT. Additional groups of males were given ectopic pituitary grafts from male or female donors. (2) differences due to the level of estrogen exposure. E-induced increases in pituitary weight and serum PRL levels was inhibited by co-administration of DHT, but not always to the levels seen in T-treated animals. Though subtle differences existed between male and female rats in the time course of these effects, DHT administration did inhibit the effects of E in both males and females when the pituitaries were under hypothalamic control. These results support our previous contention that DHT can inhibit the effects of E but that hypothalamic input is necessary for these effects to occur. In addition, our results support the view that the primary site of action of DHT is to inhibit the effect of E on lactotroph hypertrophy and hypersecretion involves maintenance of the inhibitory activity of tumor necrosis factor expressing dopamine neurons, which is typically altered during prolonged E treatment, or changes in DA receptor sensitivity at the pituitary level. Supported by BMRS Grant No. 2 S07 RR05806-11 and Research Challenge Funds from the Ohio Board of Regents.

505.8

Ultrastructural Localization of Estrogen Receptor-immunoreactivity in Guinea Pig Hypothalamus

J. D. Blaustein, J. C. Turcoite, X. Gu*, and M. N. Lehman

Neuroscience and Behavior Program and Psychology Department, University of Massachusetts, Amherst, MA 01003 and Dept. Anat. and Cell. Biol., Univ. Cincinnati Coll. Med., Cincinnati, OH 45267

We have reported previously that estrogen receptor-immunoreactivity (ER-IR) in the brain is present most abundantly in cell nuclei. However, contrary to other reports, using various immunohistochemical enhancement techniques, we have also detected ER-IR in the perikarya and processes of neurons. To determine the organelles with which ER-IR is associated, we developed a technique for ultrastructural analysis of ER-IR. Ovariectomized guinea pigs were perfused, brains vibratome-sectioned, and estrogen receptors immunostained (with H222 antibody) by a multiple-bridge peroxidase-diaminobenzidine technique followed by silver-gold intensification. Electron microscopic analysis revealed distribution of reaction product throughout cell nuclei and in association with a variety of cytoplasmic structures, including free ribosomes, golgi apparatus, lysosomes, and to a lesser extent, with mitochondria. Most surprisingly, ER-IR was found associated with small, clear synaptic vesicles (both round and flattened) and synaptic densities associated with some axon terminals. Although further controls are necessary, this suggests immunocytochemical localization of the sites of synthesis of the receptor, as well as previously unsuspected sites of action for estradiol.

(Supported by NIH NS 19327, NS 00970 and HD 21968)
405.9

TESTOSTERONE RESISTANCE ASSOCIATED DECLINE IN VASOPRESSIN GENE EXPRESSION IN THE BED NUCLEUS OF THE STRIA TERMINALIS. D. J. Dobie, M. A. Miller, D. M. Dorsa, Grecc, Seattle Veterans Affairs Med. Ctr. & Univ. of WA, Seattle, WA 98195.

To investigate whether testosterone (T) treatment in female subjects of high serum level of androgens (A) with diminished serum levels of estradiol (E2), we dosed 12 women with 1 mg of E2 (4 weeks) and 4 mg of T (6 weeks) or saline. The authors measured 3-month serum levels of androgens, 170E2, andestradiol. They found that the serum levels of T and E2 were decreased, whereas the serum levels of 170E2 were increased. The authors concluded that T treatment is effective in reducing serum levels of androgens and estradiol in female subjects with high levels of androgens and estradiol.

405.10

CHANGES IN c-fos EXPRESSION IN THE RAT HIPPOCAMPUS AFTER MANIPULATIONS OF THE BRAIN-PIGMENTARY-GLANDAL AXIS. L. Jennes, Department of Anatomy, Wright State University, School of Medicine, Dayton, OH 45435.

The rats hippocampus has been shown to contain specific receptors for estradiol and glucocorticoids. In addition, there are a number of studies that have shown that hippocampal cell proliferation is affected by estradiol and glucocorticoids. In this study, the authors investigated the effects of estradiol and glucocorticoids on c-fos expression in the rat hippocampus. They found that estradiol and glucocorticoids had opposite effects on c-fos expression, with estradiol increasing c-fos expression and glucocorticoids decreasing it.

405.11

REGULATION OF HIPPOCAMPAL GLUTAMATE RECEPTOR mRNA EXPRESSION IN RATS. Jonathan Seidman and George Price, Dept. of Medicine, Western General Hospital and MRC Brain Metabolism Unit, Edinburgh, EH12 9LX, UK.

Corticosteroids bind to hippocampal glutamate receptors (GR) and mineralocorticoid receptors (MR), thereby affecting mood and neurotransmission. There is an extensive literature on the effects of 5,7-dinoradrenochrome (5,7-DNR) on the hippocampus. In this study, the authors investigated the effects of 5,7-DNR on glutamate receptor mRNA expression in the hippocampus. They found that 5,7-DNR increased the expression of GR and MR mRNAs, with a significant increase in the GR mRNA levels. These results suggest that 5,7-DNR may regulate hippocampal glutamate receptor expression in a time- and region-specific manner.

405.12


The hippocampus is a major target for glucocorticoids and mineralocorticoids. In this study, the authors investigated the expression of GR and MR receptors in the hippocampus. They found that GR and MR receptors are expressed in the hippocampus, with the highest levels of GR expression in the CA1 and CA2 regions. These results suggest that glucocorticoids and mineralocorticoids may play a role in the regulation of hippocampal function.

406.1


Although central administration of NPY readily stimulates feeding in the rat, the pattern of NPY in relation to the circulatory system is not known. In this study, the authors measured NPY concentrations and release from the PVN of adult male rats maintained on a 4-h daily food intake (1100-1500 h, lights on 0500-1700 h). After 10 days, when body weight and food intake had stabilized, two experiments were performed. Experiment 1: NPY levels were measured by RIA in 7 microdissected hypothalamic sites of rats on 1P and control rats fed ad libitum. Groups of 3 rats were killed at 1100 h and 1300 h after food presentation. Groups of control rats were killed at the same time. In sfr rats, NPY levels in the PVN only increased at 1100 h, and steadily decreased to the control range within 2 h after food presentation. Experiment 2: The pattern of PNP NPY release in vivo was correlated with brain with dynamic changes in PVN NPY concentrations. In sfr rats, NPY levels increased 230% in the PVN and decreased 28% in the hypothalamus. In control rats, NPY levels in the PVN decreased 20% in the PVN and increased 40% in the hypothalamus. These results suggest that NPY may play an important role during the feeding cycle.
Ingestive Behavior: Peptides II

**406.3**


The relative rank order of potency (ROP) of PPY, NPY and PYY 13-36 to stimulate food intake (90-min test) in nondeprived rats (n=6) following fourth ventricular injection is compared with the ROP of these peptides to compete for saturable [125I]PPYY binding sites in three of the hindbrain implicated in ingestion; the area postrema (AP), nucleus tractus solitarius (NTS), and the reticular formation (RF). We report the estradiolized ED50 of peptides to stimulate food intake and the IC50 of peptides to compete with [125I]PPYY binding sites in these three regions of the hindbrain implicated in ingestion: the area postrema (AP), nucleus tractus solitarius (NTS), and the reticular formation (RF). We report the estradiolized ED50 of peptides to stimulate food intake and the IC50 of peptides to compete with [125I]PPYY binding sites in these three regions of the hindbrain implicated in ingestion: the area postrema (AP), nucleus tractus solitarius (NTS), and the reticular formation (RF). We report the estradiolized ED50 of peptides to stimulate food intake and the IC50 of peptides to compete with [125I]PPYY binding sites in these three regions of the hindbrain implicated in ingestion: the area postrema (AP), nucleus tractus solitarius (NTS), and the reticular formation (RF).

**406.4**

**INTRAPANCREATIC GLUCAGON INFUSIONS FAIL TO REDUCE SPONTANEOUS MEAL SIZE IN HEPATIC VAGOTOMIZED RATS.** N. Geary, J. Le Sauter, U. Noth. Psychology Dept., The City Univ. NY, NY 10027.

Remotely controlled, meal-contingent intrapancreatic infusions of pancreatic glucagon reduce the size of rats' spontaneous meals both before and late in the dark phase. Glucagon administration also inhibits elicited feeding during test meals, and under some conditions, this is blocked by selective vago-cepter antagonists. To date, however, no glucagon bolus or infusion resulted in the reduction of the first spontaneous meal 9 h after dark onset. Glucagon failed to inhibit feeding after hepatic vagotomy: Meal Size (g, m = SD)

<table>
<thead>
<tr>
<th>Vehicle (n=15)</th>
<th>Vagotomized (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6 ± 0.3</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>Glucagon 2.4 ± 0.2*</td>
<td>2.7 ± 0.2</td>
</tr>
</tbody>
</table>
| *p<0.01 vs. vehicle, ANOVA & Bonferroni test
We conclude that the hepatic branch of the subdiaphragmatic vagus is necessary for the satiety effect of exogenous glucagon on spontaneous feeding late in the dark phase.

**406.5**

**BLOCKADE OF THE CENTRAL BOMESIN (BN) RECEPTORS ENHANCES FOOD INTAKE IN PREFED RATS.** Z. Meri, T.W. Moody* and D.H. Copp. Psychology & Pharmacology, Univ. of Ottawa, Ottawa, Ontario, K1N 9A5 


The dietary intake of endogenous BN-like peptides in feeding behavior has been regulated by a lack of potent and selective BN antagonist(s). We have previously shown that [125I]BN can block suppression of food intake induced by exogenous BN. In second messenger assays, [125I]BN was also antagonized by the ability to elevate cytosolic Ca+2. The current study attempted to determine if and under what conditions this antagonist would antagonize postprandial satiety. Male Sprague-Dawley rats (350 to 400g), were food deprived for 17 hr, and injected with the antagonist (0.175 u/ml) and given access to food (standard rat chow pellets) for 60 min 'test meal'. This paradigm blockade of the central BN receptors (with 0.175 u/ml) and injection of 60 min 'test meal'. In this next set of experiments, the rats were first allowed to prefer (45 min) and then administered [125I]BN, followed by presentation of the 60 min 'test meal'. In this case, blockade of the central BN receptors significantly enhanced the consumption of the 'test meal'. This increment in food intake was dose-dependent, starting at the 100 u/ml dose and reaching maximal effect at the 200 u/ml dose. In vivo autoradiographic studies revealed that [125I]BN binding with IC50 value of 100 and the binding was greatly reduced in the presence of 1 u/ml [125I]BN, particularly at various hypothalamic nuclei. This, to our knowledge, is the first report to illustrate enhancement of food intake following blockade of the central BN receptor. The precise locations that endogenous BN-like peptides may play a role in regulation of food intake. (Supported by MRC).

**406.7**

**CCK-B BUT NOT CCK-A ANTAGONIST ATTENUATES SUPPRESSION OF FOOD INTAKE BY EXOGENOUS CCK OR INTRAINTESTINAL OLEATE.** L.A. Brenner and R.C. Ritter. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

We have reported that suppression of sham feeding by intraintestinal oleate is attenuated by the CCK type A (CCK-A) receptor antagonist, MK-329, suggesting that endogenous CCK, acting at type A receptors, participates in feeding. Recent reports suggest, however, that CCK type B (CCK-B) receptors also participate in control of food intake. Therefore, we are comparing the efficacy of the CCK-B receptor antagonist (MK-329) with that of the CCK-A receptor antagonist (L-365,260) for their ability to attenuate suppression of feeding by exogenous CCK or intraintestinal oleate infusion. MK-329 at a dose of 0.37 μmol/kg abolished suppression of real feeding by intraperitoneal CCK-8 (2 μg/kg). An equivalent dose of L-365,260 had no effect on suppression of feeding by CCK-8. In confirmation of our previous findings, 1.47 μmol/kg MK-329 abolished suppression of sham feeding by intraintestinal oleate. L-365,260 at doses of 0.37, 0.74 or 1.47 μmol/kg failed to attenuate suppression of sham feeding by oleate. These data suggest that systemic injection of exogenous CCK suppresses feeding via CCK-A receptors. Likewise, our results suggest that suppression of food intake by intraintestinal oleate is mediated by endogenous CCK, acting at type A receptors.

Supported by NIH grant NS20561.

**406.8**

**COMPARATIVE EFFECTS OF CHOLECYSTOKININ (CCK-A) RECEPTOR ANTAGONIST L 364718 AND CCK-B RECEPTOR ANTAGONIST L 360250 ON SOLID FOOD INTAKE IN RATS.** RD. Reinold,, O. Varga*, T.S. Solomon. Dept Physiol and Med, Univ-Kansas Med Ich, Kansas City, KS 66103; VA Med Cent, Kansas City, MO 64128.

Cholecystokinin's (CCK) role as an important physiological satiety factor has been supported by several studies showing that the selective CCK-A receptor antagonist L364718 stimulates food intake in several species. The inhibitory effect of CCK on feeding has generally been thought to be mediated by CCK-A rather than CCK-B receptors, because L364718 blocks suppression of feeding by exogenous CCK.A, and because the selective CCK-B agonist desulfated CCK-8 has no effect on food intake when administered centrally or peripherally. It was recently reported, however, that the selective CCK-B antagonist L 365260 is 100 times more potent than L364718 for stimulating feeding and preventing satiety in partially satiated rats (I. T. Dourish et al., Science 1987). Thus, the role of CCK-B receptors in control of food intake is not clear. In the present study we compared the dose-response effects of L364718 and L365260 on food intake during the dark period in ad libitum feeding rats. Male, Sprague Dawley rats (300-400 g) were adapted to a reversed light cycle (lights off from 1000-2200 h) and provided excess ground rat chow daily at the beginning of the dark period. Rats received L364718 (0.1, 10, 100, 1000 μg/kg ip) or L365260 (0.1/1, 10, 100, 1000, 10,000 μg/kg ip) 2 h after lights off. Food intake was measured once daily. L364718 significantly stimulated 1.5-h food intake by 4%-10 at 100 μg/kg and higher doses; cumulative intake at 3.5 and 5.5 mmOLa was increased by 50% and 100%, respectively. In contrast, L365260 had no significant stimulatory effect on feeding at any dose.

**Conclusion:** These results suggest that CCK interacts with CCK-A receptors to produce satiety during the dark period in ad libitum feeding rats.
406.9
TYPE A AND B CHOLECYSTOKININ (CCK) RECEPTORS IN THE INHIBITION OF FOOD INTAKE PRODUCED BY EXOGENOUS AND ENDOGENOUS CCK. T.H. Morgan, P.J. Angelič* and P.R. McHugh, Department of Neurochemistry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.
Recent work has suggested a role for an endogenous central release of CCK acting on CCK receptors in the regulation of food intake. In an effort to investigate whether the mechanism by which peripheral CCK inhibits food intake is through the stimulation of a central CCK receptor, we have examined the ability of the A (MK-329) and B (L-365,260) CCK antagonists to block the inhibition of food intake when given at 1 hr test produced by an IP injection of 4 ug/kg of CCK-15. At 60 minutes, increasing dosages (10 - 100 ug/kg) of the type A antagonist resulted in a blockade of the inhibition of intake produced by CCK. At 60 minutes, when the effects of exogenous CCK are no longer present, 100 ug/kg of the A antagonist significantly increased glucose consumption above baseline levels. Thus, not only were the effects of exogenous CCK blocked by the A antagonist, but the A antagonist can stimulate intake in situations in which the B antagonist cannot. (Supported by DK19302)

406.11
We (McGowan, N.K., et al., Behav. Neurosci. 104:371, 1990) have shown that chronic intrahypothalamic (IH) infusion of insulin results in dose-dependent increases in body weight and food intake. In order to determine whether enhanced insulin action, or an insulin-receptive system, we infused specific insulin antibodies (IRI Ab) or 0.9% saline into the ventromedial hypothalamus in rats. Immunoglobulins received IH saline were obtained by IRI Ab (3.0 mg/kg/hr) for 1 wk. Control animals received IH saline for 2 weeks. Experimental animals' daily food intake (FI) during saline infusion was not significantly different from controls'. Experimental animals' daily FI increased during IRI Ab infusion compared to their FI under saline infusion (from 23.1±3.5 to 29.3±4.4, p<0.01). In the wk which followed IRI Ab infusion, experimental animals' FI decreased to 27.8±1.6 g/day, a level which is not significantly different either from their FI during saline infusion or from controls' (25.6±0.8 g/day). These data provide further evidence that the hypothalamus contains an insulin-sensitive system which is involved in food intake regulation.

407.1
Previous work using biased methods for synapse quantification failed to consistently demonstrate an LTP-induced increase in synaptic number which could underlie a sustained augmentation of synaptic efficacy during LTP. This failure was attributed to the use of an unbiased technique. Young adult rats were implanted with stimulating electrodes in the medial perforant path and recording electrodes in the hilus of the ipsilateral dentate gyrus. Postsynaptic responses in the fourth (coulombic) and fifth (i.e., saturable) channels were measured at the time of the baseline recording. These results showed that the induction of LTP was not accompanied by any increase in the percentage of perforated synapses in this population of synapses on dendritic shafts or spines. Further subdivision of axosynaptic synapses into perforated (with a discontinuous postsynaptic density, PSD) and nonperforated (continuous PSD) ones showed a 24% decrease in the percentage of perforated to nonperforated synapses in potentiated animals compared to unpotentiated and control rats. This alteration is similar to, though less prominent than, the synaptic restructuring described for rats kindled in kindling model of seizures (Hosoya, 1960, 407:325). It suggests that the structural synaptic modifications underlying LTP may be viewed as an initial stage of those that ultimately lead to kindling.
Supported by Grants AG 08794 from NIA and BNS-8919022 from NSF.

407.2
Sustained enhancement of synaptic efficacy characteristic of LTP could be associated with an enlargement of the postsynaptic density (PSD) which is the site of greatest concentration of postsynaptic neurotransmitter receptors and ion channels. Previous studies have provided conflicting results regarding the effect of LTP on PSD dimensions. Earlier work was based on the use of random sections in which synaptophysin (SYP) cannot be accurately identified and sampled. To circumvent these problems, we undertook a serial section study. Young adult rats were implanted with stimulating electrodes in the medial perforant path and recording electrodes in the hilus of the ipsilateral dentate gyrus. Postsynaptic responses in the fourth (coulombic) and fifth (i.e., saturable) channels were measured at the time of the baseline recording. These results showed that the induction of LTP was not accompanied by any increase in the percentage of perforated to nonperforated synapses in this population of synapses on dendritic shafts or spines. Further subdivision of axosynaptic synapses into perforated (with a discontinuous postsynaptic density, PSD) and nonperforated (continuous PSD) ones showed a 24% decrease in the percentage of perforated to nonperforated synapses in potentiated animals compared to unpotentiated and control rats. This alteration is similar to, though less prominent than, the synaptic restructuring described for rats kindled in kindling model of seizures (Hosoya, 1960, 407:325). It suggests that the structural synaptic modifications underlying LTP may be viewed as an initial stage of those that ultimately lead to kindling.
Supported by Grants AG 08794 from NIA and BNS-8919022 from NSF.

406.10
EFFECT OF CHOLECYSTOKININ (CCK) ON GASTRIC PRESSURE AND REPORT OF GASTRIC FULLNESS IN WOMEN. P.M. Mottet, M.R. Kissilef* and P.J. Taborsky, Obesity Research Center, St. Luke's Roosevelt Hospital & Columbia University.
CCK-8 may affect food intake by augmenting neural activity from the distended stomach, thereby amplifying satiety signals (Maurasnhain, N. et al., Physiol. & Behav. 46:645-649, 1988). In order to test the hypothesis that gastric pressure in amplifying satiety signals, a gastric balloon was inflated in the stomach of each of four women with CCK-8 (112 ng/min) or saline infusion. Balloons were inflated to 500 ml or re-inflated by the subject. When the balloon was inflated to 500 ml, there were no differences in gastric pressure between the CCK-8 and saline conditions. Nonetheless, rats with fulfillment of CCK-8 administration (5.75) than with saline administration (4.5). Expectedly, the volume tolerated by CCK-8 was slightly larger than with saline infusion (695 ml). The pressure rise, however, was significantly smaller with CCK-8 (8.0 cm) than with saline (11.8 cm). The slope of the relation between fullness ratings and gastric pressure was significantly steeper when CCK-8 was infused. In addition, gastric contractions were practically abolished with CCK-8 infusion. CCK-8 appears to relax the stomach and at the same time sensitizes it to gastric pressure.

Urethane anesthesia is commonly used in studies of LTP, but it blocks amygdala kindling (Life Sci, 1989, 44:1201). In order to study the relation between LTP, kindling and kindling-induced potentiation, we administered urethane (1.5 or 0.5 g/kg) to rats that carried perforant path (PP) stimulating and dentate hilus recording electrodes. Urethane had a strong after stimulating effect on LTP of the pop spike (p<0.005), reducing LTP to <30% of that of saline controls. There was no significant difference in urethane. Urethane had a dose-related suppression effect on growth of 4 A's subsequently elicited at hourly intervals in the same rat by stimulation of the PP. The 1.5 g/kg rate failed to exhibit any partial kindling. Partial kindling failed to augment the pop spike and had a significant suppressant effect on the amplitude of the previously potentiated pop spike in the control rats. These data suggest a dissociation between the effect of urethane on LTP and partial kindling in the same brain structure. Supported by NSERC/Canada.


Anoxia grossly disrupts cognitive function, and can produce retroactive amnesia. It was therefore of interest to see how anoxia affects long-term potentiation (LTP) in hippocampus. In experiments on slices from Syngagnasawley rats, EPSP fields were evoked in areas CA1, CA3 and the dentate gyrus by stimulating the appropriate afferent pathways. LTP was elicited by two brief tetani (10 Hz for 1 s, separated by 20 s). It was manifested by a near 50% enhancement of the EPSP field, which persisted during further periods of recording of at least 30-60 min. Anoxia (N<sub>2</sub> was substituted for O<sub>2</sub> in the aerating gas) typically lasted 3 min, long enough for nearly complete (>90%), but reversible, suppression of synaptic responses. When tetanic stimulation was applied during anoxia, LTP consistently failed to develop. But if anoxia was applied 5-30 min after the end of tetanic stimulation when LTP had appeared - the temporary suppression of EPSPs was followed by a return to the potentiated level. Findings were similar in all three regions, CA1, CA3 and the dentate gyrus.

Supported by Medical Research Council of Canada.


Previous studies have shown that H<sub>2</sub>O<sub>2</sub> (0.005%) generates free radicals in slices of hippocampus and impairs synaptic transmission and spike generation. In the present study, we evaluated the possibility of sensitive long-term potentiation to free radical damage. A bipolar stimulating electrode was positioned in the stratum radiatum of hippocampal slices. Population spikes (PS) and synaptic potentials (pPS) were recorded from s. pyramidal and s. radiatum of field CA1. LTP was produced by theta frequency stimulation (100 Hz, 1 sec) at a stimulus strength that produced a half-maximal PS. Tissue was treated with a concentration of H<sub>2</sub>O<sub>2</sub> (0.002%) that had no apparent effects on evoked responses. In untreated slices (n=17), LTP was sustained for at least 60 min with H<sub>2</sub>O<sub>2</sub> (n=16) potentiation of both PS and pPS decayed. H<sub>2</sub>O<sub>2</sub> treatment beginning 5 min after HFS had no effect on LTP (n=9). Washout of H<sub>2</sub>O<sub>2</sub> following HFS allowed some but not total expression of LTP (n=16). Short-term potentiation (5-15 min) was unaffected by H<sub>2</sub>O<sub>2</sub> exposure. These data suggest that free radicals prevent maintenance of potentiation by interfering with a process during the induction phase of LTP.

407.6 EFFECTS OF CHRONIC ETHANOL EXPOSURE ON LONG TERM POTENTIATION IN THE CA1 REGION OF THE HIPPOCAMPUS. M.F. Trumble and B.E. Hunter. Dept. of Neuroscience, University of Florida, Gainesville, FL 32610.

Chronic ethanol treatment (CET) results in a depressed ability to induce long term potentiation (LTP) of the population spike (PS) in the CA1 region of the hippocampus. This study examined the effects of chronic ethanol (population excitatory post-synaptic potential (EPSP) of LTP). We compared the relative contribution of EPSP versus PS potentiation after CET. Ethanol-treated (E) and control (C) groups were fed a liquid diet containing either ethanol or sucrose for 28 weeks followed by an 8 week abstinence period. Hippocampal slices were prepared and extracellular field recordings obtained from area CA1 of the hippocampus. A stimulating electrode was placed in stratum radiatum (SR) and parallel recording microelectrodes were placed in SR and stratum pyramidale (SP). To study the characteristics of EPSP and PS LTP induction, we applied a series of high frequency stimulus trains of increasing duration. Stimulus strength was maintained at PS threshold. Following each conditioning train, recordings were obtained from SR and SP until EPSP and PS values returned to baseline. LTP was assessed by comparing the EPSP slope and PS amplitude after the conditioning train with the baseline values obtained in the absence of high frequency stimulation.

CET altered the relationship between EPSP and PS potentiation during induction of LTP. These results suggest that the mechanisms involved in the induction are susceptible to the toxic effects of CET. Current research is aimed at examining specific components of the induction process to determine the mechanism by which CET alters LTP.

Supported by the Veterans Administration and NIAAA AA02020.


Previous work (Dubrovsny et al."Steroids and Neural Activity: CIRH Symposium, Grenoble" 1983) revealed that adrenal steroids and their ring-A reduced metabolites can affect short as well as long-term, such as LTP, neuronal activity. The present report extends these results showing that LTP recorded from the granular cell layer of the dentate gyrus of the rat after tetanic stimulation of the perforant path, was affected as follows: 1.8-10g of dehydrocorticosterone, produced a marked decrease of the EPSP's, values and arrested the PS LTP at every time post-tetanic stimulation (PS) compared to the injection of vehicle (V). Naltraxone (5mg/kg) a-2-acetate decreased the 15' response and had no effect on the PS. Deoxycorticosterone (DOC) also decreased all PS's values while the PS was reduced in the first 30' Post-Corticosterone (DOC), decreased only the first 15' and 30' respectively. B-acetate, showed initial decrease of the EPSP and no effect on the PS. Allotetrahydro-DOC showed almost similar effect on LTP at 150 sec training conditions. Na-trifluorohydrochloride (NTI), an antagonist selective for delta opioid receptors, in quantities of 1 and 10 uM did not impair induction of LTP in either the MF or COM pathway. Preliminary results revealed a fast relief of the severe mood disturbances in five (5) patients.


Previously we reported that the opioid receptor antagonist naloxone prevents induction of money fiber (MF)-evoked, but not commissurally (COM)-evoked LTP recorded in the CA3 region, without affecting baseline responses in either pathway. In this study, we found that local application, into the CA1 of Cyprotyr, Orn, Pen amide (Ctop), an antagonist selective for mu opioid receptors, did not affect baseline field LTP responses. LTP responses evoked extracellularly in either the MF or COM afferents in pentobarbital anesthetized animals. One uM Ctop prevents induction of MF-, but not COM-, evoked LTP following two 100 Hz/sec conditioning trains. Na-trifluorohydrochloride (NTI), an antagonist selective for delta opioid receptors, in quantities of 1 and 10 uM did not impair induction of LTP in either the MF or COM pathway. These data suggest that mu, but not delta, opioid receptor activation may be required for induction of LTP at the MF-CA3, but not the COM-CA3 synapse. Mu-opioid, but not delta-opioid, receptor activation in induction of MF-CA3 LTP cannot be excluded. Supported by DAO1495, DAO5374, and the Rennie Fund.
407.9

Warner rats of both sexes, ranging in age from 18 days to adulthood were decapitated and the dissected hippocampi were sliced and maintained in vitro following standard procedures. Long-term potentiation (LTP) of CA1 responses was induced following brief tetanic stimulation (100 Hz, 1 sec) of the Schaffer collateral-commissural pathway. These responses were recorded with LTP induced in the LTP of evoked CA1 population spikes in adult (3 mn) males or females using a concentration of 10 M 17β estradiol (E2). By contrast, in 4-week-old animals of both sexes (10 M), LTP was induced readily, rapidly reversibly, and dose-dependently suppressed LTP. There was no observed suppressant effect of progesterone (10 M) or testosterone (10 M) on LTP from animals of this age. 3-week-old hippocampi from females exhibited similar suppressant effects of E2, but the steroid appeared significantly less potent in these younger animals. Tamoxifen (10 M), an anti-estrogen compound that has effects upon intracellular ER recognition sites, was without effect in diminishing the suppressant effect of E-2 (10 M) on 4-week-old females. These data support a possible age-dependent role of E-2 in modulating the efficacy of synaptic mechanisms underlying hippocampal LTP during a critical developmental period.

Supported by the Human Frontiers in Science Program.

407.11
DELTA OPIOID RECEPTOR BUT NOT NMDA RECEPTOR ACTIVATION IS REQUIRED TO INDUCE LTP OF SYNAPTIC TRANSMISSION IN THE LATERAL PERFORANT PATH. C.A. Brahms*, N.M. Wigram and B. Srabec*, Department of Physiology, U. of Bergen, N-5009 Bergen, Norway, and Life Sciences Department, G. of Toronto, Ontario M1C 1A4, Canada.

Rats were anesthetized with urethane and implanted with a stimulus electrode in either the lateral (LPP) or medial (MPP) division of the perforant path. Granule cell activation, under electrophysiological control. The fascia dentata was perfused with oxygenated Krebs via a push-pull cannula, while two attached electrodes recorded evoked potentials in the granule cell and molecular layers. Tetrodotoxin of the LPP or MPP elicited robust LTP in rats perfused with standard medium. In the MPP, trains given during perfusion with medium containing AP5 (50 µM), a NMDA receptor blocker, failed to produce LTP. In the LPP, however, AP5 prevented population spike potentiation without affecting the synaptic, EPSP component of LTP. Further, the opioid receptor antagonists naloxone and ICI 174,864 (delta selective; 0.1 µM) while having no effect on single-pulse transmission blocked both EPSP and population spike LTP of the LPP. The mechanism of LTP in the LPP illustrates peptidergic control of activity-dependent synaptic plasticity in brain.

407.13

Tetanic stimulation of stratum radiatum in guinea pig hippocampal slices induces a long-term potentiation (LTP) of the population spike recorded in the CA1 region. In the present study the ability of saccharin to interfere with this induction was examined. When tested in dose ranges of 2.5 to 20 µM (applied for 5-10 min, n=6 for each dose), Na saccharin did not significantly alter the size of the population spike. When the drug (10 mM, 10 min) was applied during the tetanic stimulation (400 Hz, 0.5 s) of stratum radiatum, LTP was not observed (population spike control size: 1.11 ± 0.33 µV, 30 min after tetanus: 1.13 ± 0.47 µV, n=6). In the same slices, a subsequent tetanus given in the absence of saccharin clearly caused LTP (population spike 30 min post-tetanus: 2.84 ± 0.44 µV). The amplitude of the EPSP in Mg-free medium containing CNQX (10 µM, n=4) was not significantly altered by saccharin (10 mM). In a medium containing 2-amino-5-phosphonovalerate (APV, 25 µM, n=4), however, the EPSP was slightly decreased during saccharin application. These results indicate that the blockade of the induction of LTP by saccharin is not through an antagonism at the NMDA receptors. (Supported by grants from the Canadian MRC and Glaxo Research Labs.)

407.14

Fluid samples collected during a tetanic stimulation of the rabbit neocortical surface, cause LTP in guinea pig hippocampal slices. In the present study, these samples were separated into two fractions of saccharin to interfere with molecular weights. Applications of 2 µl of <3, 3-10 or >50 kDa, but not 10-25 or 25-50 kDa, fractions on guinea pig hippocampal slices induced LTP (stratum radiatum stimulation-induced population spike in the CA1 area recorded 60 min after drug application as a % of control: <3 kDa: 127.75 ± 10.65, n=10; 3-10 kDa: 136.87 ± 9.11, n=8; 10-25 kDa: 93.13 ± 20.45, n=8; 25-50 kDa: 101.38 ± 9.99, n=6; >50 kDa: 155.25 ± 10.25, n=4). The EPSP was potentiated without a significant change in the membrane potential and input resistance (n=6). 2-Amino-5-phosphonovalerate (25 µM) did not block the induction of LTP by these substances (n=6). Gel electrophoresis (1 & 2D) of the samples revealed the presence of peptides. Samples collected from >50 kDa fractions had a potency of 25-50 µM for these samples did not induce LTP (n=6). Tetanic stimulation in hippocampal slices, however, induced LTP in the presence of samples from rabbits pretreated with MK-801 (n=6). These results suggest that the release, but not the LTP-inducing effect, of the endogenous substances involves NMDA receptor activation. (Supported by grants from the Canadian MRC and Glaxo Res. Labs.)
SYNAPTIC PLASTICITY IN THE AVIAN HIPPOCAMPUS: A. Kilaraszko, C.F. Ball, Department of Biology and Department of Psychology, Boston College, Chestnut Hill, MA 02167.

Long-term potentiation (LTP) is a stimulus-evoked, persistent increase in synaptic efficiency. LTP, which has been considered to be a neuronal mechanism underlying memory is most pronounced in the hippocampus. Although there has been recently a great deal of interest in the hippocampus and memory in birds, LTP has not yet been described in the avian hippocampus. We present here studies on synaptic plasticity and LTP in the avian hippocampus. Extracellular recordings from hippocampal slices of female song sparrows (Melospiza melodia) were taken using standard techniques. The evoked responses were much smaller and less stable as compared to those found in the mammalian hippocampus. These responses were calcium-dependent proving their synaptic origin.

Frequency potentiation could not be demonstrated. Pair-pulse facilitation showed inhibition of the second response at 20 ms and facilitation at 30 ms. Train stimulation with 20 Hz evoked a stable increase in the size of the evoked response lasting approximately two hours. This increase was classified by us as LTP. These data indicate that the avian hippocampus possesses several properties typical for the mammalian hippocampus including a most intriguing one—LTP.

Supported by NSF grant BNS 8604955 and NIH grant NS 27866-01.

407.3

ONSET OF REACTIVE ASTROGLIOSIS IN THE LATERAL GENICULATE NUCLEUS (LGN) OF INFANT AND ADULT CATS. R.E. Kalil, Center for Neuroscience, Univ. of WI, Madison, WI 53706.

Following a lesion of visual cortex, LGN neurons undergo retrograde degeneration, which is accompanied by a series of reactions in local astrocytes, known as reactive astrogliosis. One outcome of this reaction is that astrocytes become phagocytic and remove neuronal debris. Previously, we (Kalil et al., ’89) compared the time course of reactive gliosis at birth or in adulthood by using a monoclonal antibody against glial fibrillary acidic protein (GFAP) to monitor the intensity of the gliotic reaction at survival times ranging from 1 day to 26 wks. This work now has been extended by culturing GFAP-immunocytochemistry with electron microscopy to focus on the earliest reactions of LGN neurons and astrocytes to a unilateral lesion of visual cortex made in newborn or adults. In neonates, GFAP+ astrocytes are evident in the LGN at 1 day postlesion; neurons in advanced degeneration are seen at 3 days. However, this sequence possibly is confounded by the fact that LGN astrocytes on the unoperated side of the brain also are GFAP+ in neonates. By contrast, responses in adult cats rarely are positive for GFAP, but display strong immunoreactivity at 1 day postlesion, before degenerating neurons are observed. These results suggest, therefore, that reactive astrogliosis in the LGN may be triggered by a signal that precedes the onset of overt neuronal degeneration.

408.1

MORPHOMETRIC ANALYSIS OF THE CEREBELLAR NUCLEI IN LURCHER MUTANT MICE. J.A. Heckroth, Terre Haute Center for Medical Education, Indiana University, Terre Haute, IN 47809.

Mean gene expression in lurcher mutant mice causes a postnatal cell death of the entire population of cerebellar Purkinje cells. The cerebellar nuclei, while reduced in size, are reported to contain a normal number of neurons (Cody and Stance, 1977). Pre Thal. Soc. 1:1 and II, 285 (97). The volume of the cerebellar nuclei in normal and lurcher mice has been estimated utilizing plasmonic measurements of evenly spaced coronal sections. In both normal and lurcher the interposed nucleus comprises about 50% of the total nuclear volume, with the fastigial and dentate nuclei representing about 25% each. The lurcher fastigial and interposed nuclei are each reduced in volume by about 65%, while the dentate nucleus is reduced by only 53%. The volume fraction (Vn) of the nuclei occupied by neuronal somata and by neuropil has been estimated by the stereological point counting method. Neuronal somata occupy about 10% of the volume of the normal interposed and fastigial nuclei, and about 20% of the dentate nuclei. The volume fraction, combined with the total volume estimates, suggests that in the lurcher interposed and fastigial nuclei the total volume of neuronal somata is reduced by about 25% from normal, while the volume of neuropil is reduced by about 75% from normal. The relatively large reduction in the volume of neuropil may be attributed to the absence of Purkinje cell axons and terminals, and a reduction in the dendritic arbors of nuclear neurons. The neuronal volume reduction is apparently the result of smaller neuronal somata in lurcher. Further stereological measurements to determine the individual contributions of axonal, terminal, and dendritic losses to the total reduction of the lurcher cerebellar nuclear neuropil are underway.

408.4

RESPONSES OF BRAIN MACROPHAGES TO INDUCED CELL DEATH IN THE VISUAL SYSTEM OF NEONATE AND ADULT RATS. C.M. Milligan, P.Levitt and T.J.Cunningham, Dept. of Anatomy, Medical College of PA, Philadelphia, PA 19129.

The response of brain macrophages (BMOs) to induced cell death was examined in neonate and adult Long Evans rats. Lesions to the visual cortex of infant and adult rats induce cell death in the ipsilateral dorsal lateral geniculate nucleus (dLGN). BMOs are localized using the ED1 monoclonal antibody antigen, which recognizes all cells of the macrophage/macrophage lineage, but not microglia. At no time throughout normal postnatal development or in the normal adult are BMOs localized in the dLGN. After infant lesions, the entire nucleus degenerates completely by four days. BMOs are first observed among numerous pyknotic cells 48 hours following the lesion. They are large round cells and reach maximum levels four days after the lesion, completely occupying the nucleus. In adults, similar lesions to the visual cortex result in a much more protracted period of degeneration. Furthermore, subpopulations of cells located in different regions of the nucleus degenerate at different rates. The timing of appearance and location of BMOs in the dLGN correspond to the spatiotemporal pattern of degeneration in the nucleus. However, the cells are fusiform shaped and highly branched, unlike those found after infant lesions. These results suggest that the timing of BMO invasion is strictly controlled by degenerating elements. The acquisition of distinct morphologies may reflect the constraints imposed by the brain parenchyma on their invasion at different ages. (Supported by NS16487 from NINICDS)
408.5 STUDIES RELATED TO THE USE OF COLCHICINE AS A NEUROTOXIN IN THE SEPTOHIPPOCAMPAL CHOLINERGIC SYSTEM: I. SPECIFICITY OF ACTION. G. L. McAllister, Dept. of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858.

Previous research has indicated that, in the medial septum, colchicine is toxic to neurons immunoreactive for choline acetyltransferase (ChAT), but not to glutamic acid decarboxylase (GAD). In addition, a profound deficit in acetylcholinergic and cholinergic fibers is observed in the hippocampal formation, especially in the dentate gyrus and the temporal pole of the hippocampus. Here we report the results of several studies designed to more fully elucidate toxic specificity for cholinergeic and non-cholinergeic systems. (Supported by a grant from the National Institute on Aging.)

408.6 STUDIES RELATED TO THE USE OF COLCHICINE AS A NEUROTOXIN IN THE SEPTOHIPPOCAMPAL CHOLINERGIC SYSTEM: II. MECHANISM OF ACTION. G. L. McAllister, Dept. of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858.

Colchicine disrupts microtubules by binding to tubulin, resulting in the breakdown of axoplasmic transport. To determine if this mechanism of action mediates colchicine-induced toxicity of septohippocampal cholinergic neurons, the fluorescent dye Fluorescein di-Fluoromethyl-β-D-galactopyranoside (FD-FDG) was injected into the lateral ventricle of the ventral hippocampus. Thirty minutes later 2.5 μL of 0.4% colchicine or its inactive isomer, lumicolchicine, were injected into the lateral ventricle. One week later brains were fixed by perfusion with paraformaldehyde and cut on a sliding microtome through the septum and hippocampus. Both FD and ChAT-labeled cells were significantly reduced in the septal and medial septum following colchicine injections, but were not affected by lumicolchicine, indicating that colchicine blocks axoplasmic transport in septohippocampal neurons, and that this loss of transport is related to the toxic effect. Further studies are in progress to determine if the transport of nerve growth factor (NGF) is mediated. We have previously shown that NGF levels increase 200% over controls 2 weeks following colchicine injections (ELISA of NGF conducted by Dr. K.A. Crickshank at the Univ. Cincinnati Med. Ctr.). As an additional test of this hypothesis, NGF was injected bilaterally into 2 sites in each hippocampus and 2.5 μL 0.4% colchicine was injected into the lateral ventricle. Twenty-four hours later paraformaldehyde-fixed brains were embedded in paraplast, cut on a rotary microtome and processed for autoradiography. The number of radioautographed neurons was dramatically reduced in the septal and medial septum ipsilateral to the injection indicating that colchicine blocks the transport of NGF. Thus, these studies suggest that colchicine is toxic to septohippocampal cholinergic neurons because it blocks the axoplasmic transport of nerve growth factor.


Acute injuries or degenerative diseases of the CNS result in the structural degradation of damaged neurons, and synaptic reorganization of surviving neurons. These morphological changes have been attributed to the activity of proteases in the CNS. For example, the calcium-dependent proteolysis of brain spectrin (spectrin) by calpain has been proposed to constitute a requisite step in the intracellular cascade by which excitatory amino acids induce neuronal death. In order to evaluate the possible role of spectrin proteolysis in the degeneration of septal neuronal or axons after transection of the fimbria-fornix, we have used immunoblot analysis to measure spectrin breakdown in homogenates from septum and hippocampus at 1, 7 and 14 days after transection of the fimbria-fornix. Spectrin breakdown products were detected in the septum, but not hippocampus, at 7 and 14 days after transection. Western blot analysis of spectrin breakdown products in homogenates of septum and hippocampus following transection of the fimbria-fornix revealed that a 2032 larger, but not 20-322 larger, form of spectrin is detected by the polyclonal antibody in the septal cortex when compared to the controlateral cortex. These findings suggest that proteolysis of this cytoskeletal protein may be an early event in the cascade that ultimately leads to the destruction of these neurons in the absence of NGF. We are currently evaluating the effects of NGF treatments on the appearance of spectrin breakdown in the septum after fimbria-fornix transection.


As a sequel to our previous descriptive analyses, the present study has quantified the effects of ventriculosagittal myelography on the immature cerebral cortex. Hydrocephalus was induced in 4-11 day old kittens by intracerebral injection of kaolin; some hydrocephalic animals received ventriculoperitoneal shunts. At 10-14 days post-injection, hydrocephalic animals (n=4) were sacrificed at 16-44 days post-kaolin and shunted animals (n=3) at 11-30 days post-shunt. Histologically stained sections from cortical areas 17 and 22 were analyzed light microscopically with Biopquant software. Compared to saline-injected controls (n=5), cortical thickness was increased over hydrocephalic decreased 80% and 70% in areas 17 and 22, respectively. The number of neurons per 10 μm² increased 27-33% in laminae V-VI and 12-59% in layers II-IV. A consistent 35-48% decrease in soma size was most pronounced in layers V-VI. After shunting cortical thickness and soma size returned to near or above control levels; neuronal density remained either to control levels or remained about 14% lower. More improvement was found in area 17 than 22. These results indicate that hydrocephalus causes severe alterations in cortical cytoLOGY but that shunting can promote neuronal recovery. Supported by HD01257 to JPM.

408.9 MORPHOLOGY OF GAUDE NUCLEUS CHANGES AHEAD OF PRENATAL UNILATERAL NEOCORTICAL LESION. L.B. Loopstra, J.R. Villablanca and D.A. Hovda, Mental Retardation Research Center, UCLA, Los Angeles, CA 90024-1759.

4 Fetal cats (43-48) received a small lesion in frontal (3x) or parietal (1x) cortex and survived for several days or adults. Coronal, thionin stained sections of these brains were used for calculation of caudate volume, determination of neuron density and number per mm² and measurement of surface areas of areas of somatic as compared to mean computed stereometrically. Compared to weight matched controls (N=2), the volume of the caudate was larger in the lesion than the controlateral caudate was 20-322 larger. The cell packing density was similar in both sides, whereas the thionin staining of the caudate cell bodies is larger in volume than in the thalamus and cortex. (Soc Neurosci Abstr 13(87):1116). Grants HD-05395, HD-04612.

408.10 ANGIOEDEMA TRANSPORT IN ANOMITIZED CORCPSRIONAL NEURONS. M.K. Garver, B.L. McBride and K.B. Farington, Dept. of Neurology, Medical College of Georgia and Dept. of Veterans Affairs Med. Ctr., Augusta, GA 30901.

We measured slow and fast anterograde transport in proximal corticospinal axons 5, 10, and 20 weeks after complete T9 spinal cord transection as young adults. Coronal, thionin stained sections of these brains were used for calculation of caudate volume, determination of neuron density and number per mm² and measurement of surface areas of areas of somatic as compared to mean computer stereometrically. Compared to weight matched controls (N=2), the volume of the caudate was larger in the lesion than the controlateral caudate. The increase in caudate volume with preservation of cell packing density means that the caudate contains a larger amount of cells. It contrasts with the shrinkage in volume of thalamus and cortex. (Soc Neurosci Abstr 13(87):1116). Grants HD-05395, HD-04612.

One year after a T-9 spinal cord transection, fewer corticospinal neurons were retrogradely labeled with Fluoro-Gold from the T-1 level than in control rats. To determine if a similar decrease occurs in axotomized rubrospinal neurons, we assessed the number and size of red nucleus neurons which could be labeled with Fluoro-Gold. We transected the spinal cord at T-9 in ten seven-week-old female rats. At 1 year after transection, a cotton pellet soaked with Fluoro-Gold into a new transaction site at T-1. Four days later, the rats were perfused. Non-transsected matched controls were treated similarly. The red nucleus was topographically organized, with the caudal portion projecting primarily to the lumbar spinal cord. We found a significant decrease (p<0.05) in the number of Fluoro-Gold-labeled neurons in the caudal part of the nucleus in transected rats compared to controls, but no significant change in the rostral part. Labeled neurons rostrally were 19% smaller in transected rats; caudally, neurons were 40% smaller. The decrease in number of rubrospinal neurons labeled with Fluoro-Gold is comparable in magnitude to the decrease in labeled corticospinal neurons. Supported by DVA and MCG.

408.13 DEATH BY DEAFFERENTATION: THE ROLE OF MITOCHONDRIA IN AUDITORY NEURON SURVIVAL FOLLOWING COCHLEA REMOVAL. C. E. Hyde and D. Durham. Departments of Otolaryngology and Biological Structure, RL-30, University of Washington, Seattle, WA 98195.

Removal of excitatory input to second-order auditory neurons (n. magnocellularis, NM) in the chick brain stem results in the death of ~20% of the neurons at 5 days survival. In a study of ultrastructural changes in NM neurons following cochlea removal, we found a dramatic proliferation of mitochondria in the majority of ipsilateral NM neurons (Hyde & Durham, Soc. Neurosci. Abstr., 1990). However, we found a subpopulation of NM neurons with early degenerative changes which did not show mitochondrial proliferation. To test the hypothesis that mitochondrial prolifération is necessary to neuronal survival following cochlea removal, we used chloramphenicol, a mitochondrial protein synthesis inhibitor, to block mitochondrial proliferation after cochlea removal in 2-week-old chicks. We administered the rate of 1000 mg/kg/day for either 6, 12, or 24 hours following cochlea removal. After 5 days survival, animals were deeply anesthetized and sacrificed. NM neuron counts were made in 10μm Nissl-stained parasagittal sections; the NM neurons contralateral to the cochlea removal serving as a within-animal control. In animals treated 6 hours with chloramphenicol, we found no enhanced neuronal death ipsilateral to cochlea removal compared to vehicle-treated animals (p>0.60). However, in animals treated for either 12 or 24 hours with chloramphenicol, we found a significant increase in ipsilateral neuronal death (65% loss, p<0.05). Thus, we conclude that mitochondrial proliferation becomes critical to auditory neuron survival between 6 and 12 hours after the excitatory input. (Supported by PHS grants DC00018 and DC00520 and the Deafness Research Foundation)

408.14 PREDICTING INDIVIDUAL VARIATIONS IN DEGENERATION OF HIPPOCAMPAL DENTATE NEURONS FOLLOWING ADRENALECTOMY. Edward J. Roy, Diane M. Lynn,* and Charles W. Benn®. Psychology Dept., University of Illinois, Champaign, IL 61820.

Corticosterone appears to have two markedly different effects on cells of the hippocampus in rats. On one hand, elevated levels of corticosterone contribute to the degeneration of pyramidal cells. On the other hand, elimination of corticosterone by adrenalectomy may cause degeneration of dentate granule cells (Sloviter et al., 1989). However, the latter response is highly variable. Low levels of corticosterone removal are necessary adrenarchal tissue not detectable by radioummunassay may provide sufficient hormone to maintain granule cell viability. We describe simple measures that predict which individual adrenalectomized rats have degeneration of the granule cell layer. Body weight gain after adrenalectomy is correlated with granule cell layer area at sacrifice three months after surgery (r = 0.747, n = 15, p < 0.001). Also, short term loss of body weight when saline drinking water is replaced with tap water predicts the degree of degeneration of the granule cell layer (r = 0.642, n = 15, p < 0.001). The maximal effect we observed was a 64% reduction in the area of the granule cell layer. These observations may aid further study of this striking effect of adrenal hormones on brain anatomy.

DEVELOPMENT AND PLASTICITY—VISUAL SYSTEM: MOLECULAR AND CELLULAR MECHANISMS II


We are examining how a cortical area of one sensory modality might process sensory input of another modality, by routing visual input to primary auditory cortex (AI) in ferrets. Normal ferrets that are deprived of visual input by early enucleation. We have recorded electrophysiologically the visual response properties of single cells in AI of lesioned animals reared to adulthood, and compared in detail the responses with those in primary visual cortex (VI) of normal animals. Visual units in AI have longer latencies to optic chiasm stimulation (n=30, T=7.0; mean±SEM) compared to controls (n=22, T=3.9). A further experiment with our previous observation that neural input to the auditory pathway arises primarily from retinal W cells (Sur et al., Science 242:1437, 1988). Visual units have larger receptive fields in AI than those in VI (R=6.7) and generate poorer responses. Ocular dominance in both AI (n=57) and AI (n=83) is skewed towards the contralateral eye. The proportions in AI of simple (22%), complex (28%), and non-oriented (40%) cells are similar in general to those in VI. Of a smaller population of cells (n=17 in AI, 15 in VI) examined quantitatively, the degree of orientation tuning and orientation, as is the degree of direction tuning.

These results demonstrate that following specific neonatal lesions, auditory cortex can produce visual cortical receptive field properties similar to those found in normal visual cortex. Our data are consistent with the interpretation that sensory cortices may subserve some of the same sensory-motor functions as those found in visual cortex. The data suggest that the normal visual response transformations on the input they receive, or perhaps that modality-specific circuits can be induced in cortex by afferents during development.

Supported by EY07719, the McKnight Foundation, and a Whitaker Fellowship.


Previous studies have demonstrated that experimentally induced strabismus causes dramatic alterations in cortical ocular dominance in adult monkeys. When monocular stimulation techniques are employed, the majority of cortical neurons respond exclusively to one eye. However, strabismic humans exhibit robust inhibitory binocular interactions. The present study examines if an inhibitory response could be revealed in the striate cortices of strabismic monkeys when stimuli are presented to both eyes simultaneously. Extracellular, microelectrode recording techniques were used to investigate the sensitivity of cortical neurons to the relative binocular spatial phase of dichoptic stimuli (Ohzawa and Freeman, 1986). In normal monkeys (n=6), the responses of simple cells varied systematically with relative spatial phase; the disparity tuning functions typically included both excitatory and inhibitory responses. Some complex cells also exhibited phase specific responses. However, in most cases complex cells were not selective for relative binocular phase, but their binocular interactions were generally larger than either monocular response. Our studies of monkeys reared with an optically induced strabismus (n=3), revealed two major differences. In addition to an increase in the number of neurons that failed to show any binocular interactions, there was a substantial increase in the proportion of neurons that demonstrated inhibitory responses under binocular conditions at all spatial phases.
409.3

**DEVELOPMENT OF NEURAL CONNECTIONS BETWEEN TRANSPLANTED LATERAL GENICULATE NUCLEI AND HOST VISUAL CORTEX IN THE RAT.** T. Kurotsch, N. Yamamoto, and K. Tomita. Department of Physiology, Kyorin University School of Medicine, Kashiwanoha 607, Kashiwa, Japan.

Development of neural connections between the transplanted lateral geniculate nuclei (LGN) and the neuronal layers of the visual cortex (VC) was studied electrophysiologically in VC slices including transplanted LGN. Current source density (CSD) analysis of the field potentials and study of intracellular responses in VC under LGN stimulation indicated that monosynaptic connections from LGN to VC cells were established broadly in layers II-VI, while polysynaptic connections remained poorly developed in preparations of 1 week after transplantation. LGN stimulation evoked synchronous responses in a large fraction (59%) of VC cells in layers II-VI. In preparations of more than 3 weeks after transplantation, monosynaptic connections were restricted to layers IV and VI, while polysynaptic connections appeared in layers II-III and V. Efficient cells projecting to LGN became less numerous (18%) and restricted to layers V and VI. Corresponding results were obtained by morphological study using anterograde and retrograde labelling with a fluorescent dye (DiI, Molecular probe). In summary, development of afferent and efferent connectivity in transplant preparations was characterized by formation of transintrinsic projections and succeeding selection of the appropriate connections.

409.4


Long-term potentiation (LTP) or depression (LTD) of visual cortical responses to extracellular (extracortical) afferent stimuli can be induced by tetanic stimulation of ON of the other side, respectively, in kittens during “critical period” of postnatal development (Tsumoto and Suda, Brain Res., 168, 190, 1979). In the present study, we addressed a question of what conditions are optimal to induce such homosynaptic LTP and heterosynaptic LTD, in 4-8 week-old kittens anesthetized with 5% and Halothane. Extracellular field potentials to test shocks applied alternatively to each optic nerve at 0.1 Hz were recorded simultaneously from the primary visual cortex of both hemispheres. Tetanic stimulation applied to ON at 5 Hz for 1 or 5 min failed to induce LTP but longer tetanus (15-60 min) could induce LTD and LTD. Since afferent spontaneous activity was suggested to play a role in visual cortical plasticity (Stryker and Harris, J. Neurosci., 6, 2117, 1986), retinal neuronal activities were blocked by an intracranial injection of tetrodotoxin (TTX). After the TTX injection, homosynaptic LTD could more easily be induced but heterosynaptic LTD could not be induced at all. These results suggest that afferent spontaneous activity may play a role in induction of LTD and LTP in kitten visual cortex.

409.5

**NON-HEBBIAN SYNAPSES IN RAT VISUAL CORTEX.** A. Kostel (1), T. Kishibori (2) and B. Luck (3). (1) Friedrich-Miescher-Mitarbeit der Max-Planck Gesellschaft, (2) Max-Planck Institfor biologische Kybernetik, 74076 Tübingen, FRG.

It has been demonstrated experimentally that synaptic connections in the visual cortex can be modified by correlated activity of pre- and postsynaptic cells, as predicted by a classical model proposed by Hebb. These studies also have shown that the synaptic enhancement was restricted to only those inputs which were active simultaneously with the postsynaptic cell. We investigated whether the enhancement remains also spatially confined to only those postsynaptic regions that have been coactivated with the presynaptic fibers. Double intracellular recordings were performed in slices from the visual cortex prepared from 2-4 week old rats. The two cells were lying close to each other (distance <500 μm), but they were not synaptically connected. Stimulating electrodes were placed in the white matter and current pulses (ISI = 400-600 μs) were applied to yield subthreshold EPSPs in both cells. Synaptic enhancement was induced by pairing extracellular input to intracellular depolarization of one of the two recorded cells. The other cell was depolarized. In all 4 out of 15 experiments, in which we could potentiate the synaptic responses of the paired cell, the responses of the second cell were also reinforced. Control experiments with a third intracellular recording electrode showed that the strengthening does not extend throughout the whole slice. Our results indicate that in contrast to the Hebbian rule, synaptic enhancement is not restricted to the paired cell, but spreads to neighboring neurons. This effect could be relevant during the development of the cortex: adjacent cells tend to innervate the same set of functional properties. Thus in turn lead to clustering of cells with similar response properties, a common organizing principle of the cortex.

409.6

**SYNAPTogenesis COMPLEMENTS ASSOCIATIVE SYNAPTIC MODIFICATION IN A MODEL OF THE DEVELOPMENT OF OCULAR DOMINANCE.** C.M. Colbert, S.M. Friedmann, and W.B. Lacy Dept. of Neurosurgery, Box 450 Med. Ctr., Univ. of Virginia, Charlottesville, VA 22908.

Experience plays a central role in establishing neuronal properties and connectivity in the developing nervous system. In most activity-based neural network models, activity shaping is achieved through associative modification of existing synapses. Here we report on a model in which activity governs the formation of synapses as well as modification of existing synapses. The rules governing synaptogenesis are derived from the associative modification rules. The synaptogenesis rules are based on both physiological and anatomical investigations in a number of systems, including the neuromuscular junction, the superior cervical ganglion, the lateral geniculate nucleus, the superior colliculus, and cerebral cortex. These studies imply that distinct presynaptic and postsynaptic mechanisms, in tandem, govern synaptogenesis. Once a synapse is formed, its efficacy is governed by a form of associative synaptic modification that has been observed in the hippocampus and in visual cortex.

To verify the validity of the model, we simulated the development of ocular dominance in the kitten under normal and experimentally induced deprivation conditions including 1) strabismus, 2) monocular visual deprivation, 3) monocular visual deprivation with muscimol infused into visual cortex, and 4) monocular visual deprivation with APV infused into visual cortex. The simulations produced histograms of ocular dominance consistent with experimentally observed distributions.

Supported by NIH R01 NS51548, NIMH R01 MH00622, NIH ST32 G00726713.

409.7

**EFFECTS OF APV AND NMDA ON TECTAL CELL ACTIVITY IN XENOPUS.** W. Luck, W. Eichler* & S.B. Udin. Dept. of Physiol., SUNY, Buffalo, NY 14214.

Chronic blockade of NMDA receptors with APV prevents matching of binocular tectal maps in eye-swapped Xenopus during the critical period, and chronic infusion of NMDA restores this plasticity in postcritical period Xenopus. In order to assess whether APV or NMDA produce these effects by altering tectal cell firing activity, we have tested the output of a subset of tectal axons which project to the nucleus isthmi. We treated one tectal lobe with a drug and recorded responses to light flashes from the isolated tectal lobes in the other eye. Chronic treatment with the NMDA antagonist APV did not alter tectal cell activity in critical period Xenopus when compared to age-matched controls. Chronic treatment of postcritical period animals was found to increase tectal cell firing frequency by an average of 35% when compared to age-matched controls. No difference in tectal cell firing frequency was observed in postcritical period animals. These data indicate that the effects of APV on blocking plasticity are not due to a nonspecific decrease in visually evoked tectal cell firing. The role of NMDA receptors in the restorative effects of APV on tectal plasticity may be related to an increase in tectal sensitivity, but do not support the hypothesis that normal loss of plasticity is due to a reduction in NMDA potency. The authors thank Dr. Boxer for the Retina Screening Fellowship from the March of Dimes Birth Defects Foundation to W. J. S.

409.8

**PHYSIOLOGY AND MORPHOLOGY OF NEURONS IN RANA PIPIENS TECTAL SLICES.** P. W. Hickmott and M. Constantine-Paton. Dept. of Biology, Yale Univ., New Haven, CT 06511.

Work in our lab has implicated the N-methyl-D-aspartate (NMDA) receptor in the activity-dependent refinement of the retinotopic map in Rana pipiens. Thus we have examined the physiological effects of various drugs that interact with the NMDA receptor, on the evoked potential in R. pipiens optic tectum (Gec. Neurosci. Abstr. 15, 15’79, 1989). We have now begun to examine the responses of single cells from the tectal slice preparation.

We have successfully recorded from tectal neurons with resting potentials ranging from 20 to -70 mV, and input impedance in the 200-400 MΩ range. In response to depolarizing voltage steps, we observe large transients inward currents, followed by sustained outward currents, which correspond to action potentials. In contrast, if we stimulate in the optic tract these currents that appear to be EPSC's generated by the opening of a Cation channel with a reversal potential of around 0 mV at negative holding potentials we frequently observe large, rapid inward currents consistent with potential firing in the cell. In some cells, instead of this EPSC, we have observed a second type of PSC with a reversal potential of about -45 mV. In some cells, we find frequent, small, spontaneous currents that reverse at the same level as the PSC.

We have also directly demonstrated the presence of NMDA receptors on some cells, bath-applied APV blocks the PSC's completely and PSC's appear to be both an inward and outward current. Supporting information has been obtained from the identification of bicuculline-sensitive GABA A receptor mediated currents. Our results with APV suggest that the NMDA receptor may be a critical component of the tectal circuitry.
A monoclonal antibody against Calcium/calmodulin dependent protein kinase II (CAM II) was utilized to localize the kinase in developing kitten visual cortex. CAM II was first expressed in deeper cortical layers (V and VI) at postnatal day 0 and gradually appeared in all the other layers within the first two weeks. Expression of the kinase was low in cells of layer V and upper layer VI at about 30-40 days of age and by postnatal day 90, CAM II immunoreactivity was found in layer II, IV and deeper layer VI. This laminar pattern remained constant into adulthood. EM studies showed that the kinase was found in both pre- and post-synaptic locations, particularly on synaptic vesicles and in post-synaptic densities in adult animals. Increased number of immuno-positive neurons were found in the middle cortical layers of adult cats when geniculate input was removed by an ipsilateral thalamic lesion performed early in life. These results show that CAM II is transiently expressed in specific populations of cortical neurons during postnatal development and that the level and distribution of the kinase is use-dependent.

Several aspects of postnatal visual cortical development suggest environmentally induced changes in gene expression. Specifically, dark rearing prolongs the critical period and brief (2 day) visual exposure in dark reared animals promotes rapid physiological changes. Oncogenes provide a convenient window for studying changes in signalling pathways within the cell. The present study begins analysis of the effects of dark rearing and exposure to light on oncogene mRNA levels in normal kittens. We compared normal and dark reared cats at 5 and 20 weeks of age, and dark reared cats given 2 days of visual experience at 5 weeks. To date we have examined the expression of e-fos, c-myc, and egr-1. Levels of e-fos mRNA were decreased 40% by dark rearing at both ages. c-myc mRNA levels were elevated by dark rearing at both 50 (90%) and 20 (30%) weeks. Lesser effects were seen with egr-1, which was decreased 25% by dark rearing at both ages. Brief visual exposure in dark reared animals caused additional decreases in e-fos and egr-1 mRNA levels of 40%, whereas c-myc levels were unchanged. These environmentally induced alterations are consistent with a possible role of proto-oncoproteins in visual cortical plasticity. Analysis of additional cortical structures is underway.

409.11 PHYSIOLOGICAL ACTIVITY REGULATES zif/268 IN THE VISUAL CORTEX. P.F. Vorley, R. Christy, Y. Nakahase*, and J.M. Basabian. Dept. of Neuroscience and Deps. of Molecular Biology and Genetics, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.
In recent studies, we demonstrated synaptic activation of the transcription factor gene, zif/268, in dentate granule cells following brief tetanic stimulation of perforant path afferents leading to long-term potentiation. To address whether zif/268 is activated by physiological activity, we have examined the regulation of zif/268 in the visual cortex. In control animals, zif/268 mRNA is present at relatively high levels in layers II-IV and VI of the visual cortex. Moreover, immunostaining is prominent in nuclei of subpopulations of neurons in these layers. Visual deprivation studies indicate that these basal levels of zif/268 mRNA and protein in the visual cortex are driven by visual stimuli. Monocular injection of tetrodotoxin rapidly and selectively reduces levels of zif/268 mRNA and immunostaining in rodent primary visual cortex receiving predominant inputs from the treated but not control eye. Additionally, dark adaptation (4d) results in reversible reductions of zif/268 mRNA in visual cortex. These findings suggest that physiological synaptic activity is sufficient to elicit a robust immediate early gene response in neocortex. Accordingly, zif/268 may help orchestrate genomic responses underlying the plasticity of mature cortical neurons to altered patterns of sensory stimulation.

409.12 VISUAL EXPERIENCE REGULATES SPECIFIC SYNAPTIC MALIGNIC COMPONENTS IN THE DEVELOPING RAT STRIATE CORTEX. Aniek A. Schouws,* and Ira B. Black. Division of Developmental Neurology. Cornell Medical Center, NY 10021, and UMDS/Robert Wood Johnson Medical School, Piscatway, N.J.
To study potential modulation of synaptic molecular structure by experience, the major postsynaptic density protein (mPSDp) in rat striate cortex was measured during normal development and during a period of visual deprivation. A purified synapsosomal membrane (SM) fraction from Long-Evans hooded rats was isolated, and mPSDp (separated by SDS-PAGE) was quantitated using 125I-calmodulin. During normal development, mPSDp matures differently from other molecular synaptic components: whereas total SM protein increased 32-fold between days 5 and 60, the mPSDp increased 455-fold. Rats reared in complete darkness for 2 weeks following birth exhibited 60% of total SM protein found under normal rearing conditions (12hr light/dark cycle). Visual deprivation during a critical period that interfered with normal development. Moreover, striate cortex mPSDp was selectively affected: following dark-rearing, striate cortex mPSDp was only 34% of normal. In contrast, light-deprivation did not alter mPSDp in parietal or prefrontal cortices, indicating topographical specificity of the synaptic response. Adult striate cortical mPSDp, on the other hand, was not affected by two weeks of dark exposure. We conclude that visual experience during development selectively modified specific components of the postsynaptic membrane structure. Since the mPSDp (MW= 513kDa) is an autoradiophotoly subunit of a Ca++/calmodulin enzyme, these data raise the possibility that this kinase plays a role in neural plasticity.

The developmental localization of beta adrenergic receptors has been studied in kitten visual cortex using immunocytochemistry and autoradiography. Using a monoclonal antibody, which recognizes beta receptors and a beta-specific ligand (I-125 CYP, with 5HT competition) we have found specific populations of neurons that express beta receptors in the cortex. In adult animals, the receptors are concentrated in the superficial and deep cortical layers (layers 1, 2, 5 and 6). In neonatal kittens, fewer receptors are present, but they appear to be concentrated in the deep cortical layers and white matter. Receptor numbers increase after birth and by 14 days of age, the laminar distribution approaches that of the adult animals, with further thinning out of labelled cells in layer IV as assayed by northern blot. Autoradiographic studies in normal kittens showed that mPSDp was labelled in stellate cells, although a few pyramidal cells were stained in layers 3 and 5. This pattern of distribution is to be independent as it was not affected either by removal of thalamic input early in life, or by early interruption of adrenergic input.

Two facts led us to speculate that the density of binding sites associated with the N-methyl-D-aspartate (NMDA) receptor might vary with age in the cat visual cortex. First, the NMDA receptor has been implicated in neural plasticity. Second, plasticity in cat visual cortex varies with age. To test this notion we measured binding of the NMDA antagonist, MK-801, in homogenates of visual cortex membranes from cats aged 7 days (60), 21 d, 43 d, 83 d, 7-8 mos and over 2 years. Binding was measured in the presence of 100 uM glutamate, 30 uM glycine and 30 uM spermine after 3 hours incubation at which time equilibrium had been reached.
Binding was maximal at 43 and 83 days, decreasing in both younger and older animals. Saturation analysis showed that the variation in binding with age resulted from variation in Bmax.
Addition of glutamate, glycine and spermine caused a greater percentage increase in binding in adult animals than in 43 day old animals. This may indicate that NMDA channels have more frequent spontaneous openings in 43 day old animals than in adults.
409.15

SUBPLATE-1: A MOLECULAR MARKER FOR EXCITATORY NEURONS IN SUBPLATE ZONE OF DEVELOPING CAT CORTEX? P. Whible, J. Liljebäck, J. R. Naegelé, K. Albos. MPI Biofysik Chemie, Munich, Germany; and Dept. of Ophthalmology, Yale University School of Medicine, New Haven, CT 06510, USA

A transiently expressed antigen in the cortical subplate zone of kittens was detected with a monoclonal antibody called SUBPLATE-1. Immunohistochemical analyses, autoradiographic labeling of synaptosomes, and Western blot analysis of P10 P12 kitten cortex homogenates, a single band of 60 kD was stained. Phosphatase does not alter the staining, suggesting the epitope does not involve a phosphorylation site. SUBPLATE-1 immunoreactivity (IR) is expressed in invaginated spiny pyramids, as shown by combined intracellular injection of Lucifer yellow and immunocytochemistry. The neurons send a major dendrite toward the white matter and several minor dendrites toward the gray matter. Axons typically arise from the major dendrite and descend into the white matter forming recurrent collaterals. This morphological type is also labeled when DiI is implanted into thalamus or supragranular cortical layers of young kittens. It suggests that the SUBPLATE-1 antigen may be expressed in transient neurons of pyramidal morphology with long-range axonal projections. This concurs with double immunofluorescence studies demonstrating that the majority of SUBPLATE-1 neurons are likely to be glutamatergic or peptidergic. We speculated a glutamatergic nature, because many white matter neurons of invaginated pyramidal shape displayed gluatamate IR. Curiously, few, if any of the SUBPLATE-1 neurons contain aspartate or a neurotransmitter not yet identified in the subplate zone of cat cortex.

409.16

NEUROPEPTIDE-Y IMMUNOREACTIVITY (NPY-IR) AND SOMATOSTATIN-IMMUNOREACTIVITY (SOM-IR) FOLLOW DIFFERENT DEVELOPMENTAL PATTERNS IN CAT VISUAL CORTEX. Dale Hogan and Nancy E. L. Berman. Department of Anatomy and Cell Biology, Univ. of Kansas Medical Center, Kansas City KS, 66103.

We examined the numbers and location of NPY-ir and SOM-ir cells in postnatal kittens and adult cats to determine whether the expression of these peptides differs developmentally across visual cortical areas. The total number of NPY-ir neurons per section increases postnatay in all areas examined. Adult visual cortex has three times as many cells as the newborn. The density of cells remains the same throughout development, the increase in number being directly correlated to the increasing brain size. The increase in NPY-ir cell numbers is accompanied by an increase in NPY-ir axons. Developmentally, medial areas are invaded by immunoreactive processes first, with the lateral areas lagging about a week behind. As the axons grow into the cortex, they follow a radial pattern, growing straight up through the cortical plate and branching first in layer 1. In the adult, dense fibers crisscross the whole extent of the cortex in secondary visual areas. In areas 17 and 18, stained processes avoid layer IV.

By contrast, SOM-ir cells has reaches the adult pattern. These results implicate somatostatin as a possible factor in guidance of axons into secondary visual areas, but do not support such a role for neuropeptide-Y. (Supported by MH33399, BNS881997, and RCD8954894)

NERVE GROWTH FACTORS VII

410.1


It has been postulated that NGF binding to the low affinity-fast dissociating NGF on Schwann cells is involved with neurite extension during development and regeneration. Since central projections of somatosensory fibers sprout into the spinal cord after neonatal administration of antibodies to NGF (ANTI-NGF), it is of interest to determine if the distribution of NGFR correlates with the occurrence of sprouting. Neonatal rats were given daily injections of ANTI-NGF (3 μg/gm body wt.) for a period of up to one month. Treated and untreated rats were sacrificed on postnatal days (PD) 0, 7, and one month. The NGFR distribution was determined using the monoclonal antibody 192 and standard immunohistochemical techniques. In the untreated rats, NGFR distribution on PD 0 and 7 was localized in laminae I, II, III and medial IV; fasciculus cuneatus and gracilis tracts above and below the central canal and bundle around motorneurons. By one month of age, distribution was localized to only laminae I and II and the tracts above and below the central canal. Data from the ANTI-NGF treated rats will be presented. We hypothesize the NGFR are involved with sprouting and synaptogenesis of somatosensory components of the spinal cord.

410.2

PHASIC INCREASES IN NERVE GROWTH FACTOR OCCUR IN DORSAL ROOT GANGLIA AFTER SCAR TISSUE CRUSH. M.R. Wells and J.P. Schwartz. Nerve Regeneration Laboratory, Veterans Administration Administration, Northport, N.Y. 11787 and CNB, NINCOM, NIH, Bethesda, Md. 20292.

The nerve growth factor content of sensory ganglia was examined after nerve injury to determine its possible relationships to the metabolic response of dorsal root ganglion cells to axon injury. Male, Wistar-Furth rats were subjected to unilateral crush injuries to the sciatic nerve at the level of the sciatic notch. At 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 14, and 30 days after injury, the L5 and L6 lumbar ganglia and the C6 cervical ganglia were removed bilaterally and examined for NGF content by an enzyme-linked immunoadsorbent assay. Ganglionic NGF increased up to 500% phasically and bilaterally at days 1, 4, 7, and 6 days in the L5 and L6 ganglia combined (p<0.0002, ANOVA). The strongest effect was observed bilaterally at day 7. The C6 cervical ganglia showed an increase in the NGF content of cervical ganglia at 3 days after injury. Since sciatic nerve section is known to elevate NGF in Schwann cells, perhaps mediated by interleukin-1 released from macrophages, these results suggest the possibility that Schwann cells in the ganglia may also synthesize increased amounts of NGF. Why this increase should occur phasically remains to be determined. Phasic increases in ganglionic NGF coincided temporally with specific metabolic changes observed in identified axonized neurons in the sciatic nerve ganglia. (Wells, Exp. Matol, 95: 313, 1987). However, in these prior studies, the pattern of responses of the injured and uninjured sides differed. The source and localization of the NGF increase will be important in determining if it may play a role in the metabolism of axotomized sensory neurons. Supported by the Veterans Administration and NIH.
NERVE GROWTH FACTOR (NGF) IMMUNOREACTIVITY IS RESTRICTED TO A SUBPOPULATION OF SENSORY NEURONS: C. Byrnes, J. Voigt, R. Byrnes, C. Auffray and A. Keller. Dept. of Physiology, Flinders University, Adelaide, Australia, 5042. We have examined co-localized NGF and NGF receptor immunoreactivities in neurons of chicken peripheral ganglia using anti-peptide antisera. In dorsal root ganglia (DRG), the antigen is visible in small neurons with increasing concentrations, from embryonic day 10 up to adult ages. The antigen is also present within some neurons of trigeminal ganglia, but not in any other ganglia. Neuronal co-localized NGF receptor is detectable in neurons of the vestibulo-acoustic, nodose, petrosal, ciliary or, surprisingly, sympathetic ganglia. Neither antigen is detectable in either central or peripheral nerve processes except distal to a nerve ligation. Characterization of the immuno-reactivity present in DRG was achieved following chromatographic separation of ganglion extracts; antigen being found only in fractions that eluted identically with pure NGF. Extracts of sympathetic ganglia also contained immuno-chemically identifiable NGF. We conclude that the factor exists in different compartments of reactive and non-reactive neurons, and that only some of the NGF present can be detected immunohistochemically.

NGF and NGF Receptor Expression in Primary Regenerate Cultures Derived From the Embryonic Mouse Septum I.D. Robak1, M. Downien1, H. L. Le1, J. Zuckerk1, T. H. Langer1, U. Diam2, and B. H. Walter3. 1University of Chicago, Chicago IL 60637, Case Western Reserve University, Cleveland OH 44106, and University of Basel, Switzerland. In the adult central nervous system, nerve growth factor (NGF) is expressed at high levels in the hippocampus while its receptor (NGF-R) is synthesized in the septum, primarily by the cholinergic neurons which project to the hippocampus. In order to better understand the regulation of NGF and NGF-R biosynthesis, we have grown either hippocampal or septal cells derived from embryonic day 15 fetuses in regenerative culture. At this developmental stage, septal and hippocampal neurons have not yet formed contacts with one another. We previously reported that the in vivo developmental profile of hippocampal NGF mRNA and protein expression can be partially recapitulated in vitro by hippocampal regenerates (Robak, et al., Dev. Biol. 137:451, 1990). We now report that septal regenerates express both NGF and NGF-R. NGF protein rises steadily with time in these cultures. By day 21, septal regenerates contain an average of 40 pg NGF proteinizing total protein (ca. 50% of that seen in day 21 hippocampal regenerates). Levels of NGF mRNA are high at day 7 (11.4±1.4 arbitrary O.D. units) and day 14 (11.6±2.3), but decrease by day 21 (8.5±1.3). Levels of NGF-R mRNA rise from day 7 (4.9±2.2) to day 14 (8.3±3.1), and then decrease by day 21 (2.6±0.8). Hippocampal regenerates, on the other hand, express levels of NGF mRNA approximately 50% higher than those seen in septal regenerates, but do not express detectable NGF-R mRNA. These results demonstrate that both NGF and NGF-R are synthesized in the septum in the absence of hippocampal interactions. Moreover, NGF and NGF-R mRNA expression may be coordinately regulated in these septal cultures. In conjunction with data from other groups, this work is consistent with a model in which septal-derived NGF provides local regulation of NGF-R expression in the septum. Supported by NIH grants 5-T32-HD07009, and NS-27877.

EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR LIKE IMMUNOREACTIVITY IN THE FOREBRAIN OF POSTNATAL RATS. M. Nishizuka and Y. Arai1. Dept. of Anatomy, Juntendo Univ. Sch. Med., Tokyo, Japan. The immunohistochemical localization of developing nerve growth factor (NGF) receptor in the forebrain of postnatal rats was studied using specific anti-NGF receptor monoclonal antibody, 192-1gG. Wistar rats were deeply anesthetized with pentobarbital and perfused transcardially with a buffered parafomaldehyde at 1-10 postnatal days. The brain sections were incubated with a supernattant of hybridoma cell culture containing 192-1gG (courtesy of Prof. H. Hatanaka) and processed for ABC method. NGF receptor like immunoreactive neurons were obtained in the septum, the nucleus of the diagonal band of Broca, the ventral pallidum, and the caudate-putamen. Some rats were injected with 2.5S mouse NGF(1-2 ng/0.1 BSA) into the hypothalamus 3 days before sacrifice. The number of immunoreactive neurons increased in the ventral pallidum of NGF infused pups. Immunoreactive cells were also obtained in the arcuate nucleus of NGF infused rats. These data provide a possible explanation for our previous results that NGF facilitated the growth of transplanted hypotalamic neurons in fetal rats.

Sites of NGF and NGF mRNA Synthesis in the Developing Rat Embryo Esther Wheeler, Ph.D.1, M. B. Byrnes, Ph.D.2 and Mark Bothwell Ph.D.1. 1Dept. Physiology and Biophysics, Dept. of Anesthesiology and Biological Structure2, University of Washington, Seattle, WA 98195. The temporal and spatial expression of nerve growth factor (NGF) and its receptor (NGF-R) has been determined by in situ hybridization and immunocytochemical analysis of rat neocortices and embryos aged 12 to 22 days postconception. In addition to the expected expression in the nervous system, transcripts for both molecules were detected in a wide variety of non-neuronal cells. In the developing skin of the maxillary pad and the developing limb, NGF was localized in the epithelial and underlying mesenchymal cells. Expression of NGF co-localized with NGF-R transcripts in the peripheral cell layers of the adjacent developing epithelium. In developing teeth, NGF and NGF-R transcripts were co-localized in the mesenchymal cells of the odontoblasts and the odontoblasts themselves. NGF and NGF-R transcripts were also observed in developing myoblasts but not in fused myotubes. By contrast, NGF-R mRNA, but not NGF mRNA, was detected at high levels in the mesenchymal cells, but not the odontoblasts, of the developing incisor. These results suggest NGF and NGF-R are involved in the development of non-neuronal structures. Since both factor and receptor are expressed over the entire course of embryonic limb development in a pattern consistent with a role in the instructive mesenchymal/epithelial interactions that effect morphogenesis.

EXOGENOUS NGF REGULATES NGF RECEPTOR (NGFR) EXPRESSION IN DISCRETE CNS REGIONS. M. Fucito, F. Polato1, G. Vantini, M. Guittigno1, A. Leon. Filice Research Laboratories, 35031 Abano Terme, Italy and 1Inst. of Human Anatomy, Verona University, 37124 Verona, Italy. We recently showed that exogenous NGF can regulate in vivo the expression of NGF mRNA and corresponding protein levels in the basal forebrain cholinergic neurons. We have here evaluated the effect of exogenous NGF on NGF mRNA levels in both neuronal and non-neuronal structures expressing NGF in rats. We administered the hormone to adult rats via mini osmotic pumps (50 μg/3 weeks) and to newborn rats by repetitive injections (5 μg, every other day from postnatal day 2 to 6). NGF administration increased NGF mRNA accumulation (up to 3-fold). However, no NGF immunocytochemical changes were observed. These structures include sensory pathways, brain stem sensory nuclei and some optic-related structures. In adult animals an increase in NGF was also induced by NGF in many thalamic nuclei displaying slight NGF immunoreactivity. In adult animals, enhancement of NGF immunoreactivity was also observed in non-neuronal cells such as ependymal cells and tanyocytes. No effect of NGF was observed in spinal motor neurons and brainstem motor nuclei showing to express NGF during development. These data suggest that, within the CNS, the role of NGF is broader than traditionally thought.

AGING SYMPATHETIC NEURONS WITH ELEVATED LEVELS OF CYTOPLASMIC CALCIUM DO NOT DIE BUT BECOME ATROPHIC UPON DEPRIVATION OF NERVE GROWTH FACTOR IN VITRO: CHARACTERIZATION OF NEURONAL ATROPHY. J. Toth, S. Tanaka2 and S. Masuko. Deps. of Nat. Sci. and Anatomy, Saga Medical College, Nebeishi, Japan. Sympathetic neurons become independent of NGF for survival upon aging; trophic deprivation does not cause death of neuromuscular cells in the adult. We hypothesized that this IgG due to elevated levels of cytoplasmic free Ca(2+) (FEPAS 86:6421, 1989). Indeed, the neurons with raised levels of cytoplasmic free Ca(2+) (FEPAS 86:6421) became resistant to NGF deprivation and their survival tended to be independent of NGF(J. Cell Biochem 14:95, 1990). These neurons, however, became gradually atrophic upon withdrawal of NGF: cell shrinkage occurred slowly and neurites became thinner. No major alterations were observed in the structure of cellular organelles. This process was reversible. Thus, we can dissociate the trophic effects of NGF from neuronal damage under these conditions so that normal neurons are available for studying cellular signaling associated with atrophy and hyperplasia. Neuronal atrophy caused by NGF withdrawal appears to be partly prevented by the activation of protein kinases but not prevented by high K+ (35mM) medium.

NERVE GROWTH FACTORS VII

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

THURSDAY AM

Nerve growth factor (NGF) immunoreactivity is restricted to a subpopulation of sensory neurons.
410.9 SCIENTIFIC NERVES TRANSPLANTED INTO THE CNS EXHIBIT ELEVATED NERVE GROWTH FACTOR (NGF) PROTEIN LEVELS. R. J. Messersmith, M. Faberano, L. Mooshahi, L. J. Kromer, Dept. of Anatomy and Cell Biology and FGNC, Georgetown University, Washington, DC 20007

After bilateral fimbria-formix aspiration lesions in female adult Sprague-Dawley rats, segments of scientific nerve were inserted rostrally into the septum and caudally into the hippocampus. Previously, we demonstrated that two weeks post-transplantation there was a significant increase in the number of choline acetyltransferase-positive (ChAT+) cells in the medial septum compared to animals receiving lesions only (41% vs 21% of normal). In order to examine the role of NGF in the lesion-associated elevation in ChAT+ cells, we measured NGF protein levels using a two-site immunosay. NGF levels were measured in normal nerve segments; nerves lesioned, ligated and left in situ for 2 weeks; and nerve grafts 2 weeks post-transplantation. Septal NGF levels were measured in normal, lesion controls, and animals receiving transplants.

NGF levels in the transplanted were significantly higher than in normal nerve segments and slightly less than in lesioned nerves in situ (Mean NGF pg/mg wet tissue wt: 1.3 ± 0.2, 0.7 ± 0.1, 2.0 ± 0.2, respectively). Septal NGF levels in lesioned animals (0.3 ± 0.03) were lower than in normal rats (0.4 ± 0.03) or in specimens with transplants (0.4 ± 0.04). These results suggest that NGF released from the transplant may be one factor that returns NGF levels in the septum and supports an increase in the number of ChAT+ septal neurons after lesions. Supported by NIH grant # NS 23322.

410.10 STIMULATION OF CAROTID ACETYLTRANSFERASE IN PC-12 CELLS BY NGF: RELATIONSHIP TO CHOLINE ACETYLTRANSFERASE ACTIVITY. P. N. Schlesinger, H. L. Barone, H. D. Fabrazzo, G. Bonner, M. A. Mez, D. H. Kissel, M. L. Fabre, Department of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, N.C. 27709

When incubated with or without choline acetyltransferase (PC-12) cells, nerve growth factor (NGF) causes neurite extension, phosphorylation of kinases, and increases in certain enzymes, including choline acetyltransferase (ChAT). In the present study we have determined the effects of NGF on both ChAT and carotid acetyltransferase (ChAT). PC-12 cells were cultured in serum-free DMEM +/- NGF. Cells were periodically examined morphologically, harvested, washed, lysed, and assayed for ChAT and CarAT, using [14C]acetate - CoA and either choline or carinate as substrates.

A basal activity of CarAT in PC-12 cells was more than 100-fold greater than that of ChAT. Both enzymes were stimulated by NGF in parallel, in a concentration and time-dependent manner. Significant stimulation was observed by 24 hr incubation with 5 ng/ml of NGF, and both activities were at least 3-fold higher after 72 hr incubation. Additional experiments showed that 10 µM or more of acetylcarnitine stimulated ChAT activity in the absence of NGF. These observations suggest that the activities of CarAT and ChAT may be coupled and that their stimulation by NGF may favor a reversal of cholinergic deficits in animal models of neuronal degeneration.

410.11 NEUROGENIC EFFECT OF RECOMBINANT HUMAN GROWTH FACTOR (rhNGF) IN VITRO AND IN VIVO: COMPARISON WITH THE EFFECT OF MOUSE NGF (mNGF). M. Kakinaka*, M. Iwane*, N. Nakahama*, M. Suno, Bio-Tech Labs, 930 Division, Takeda Chem. Ind., Ltd., Josuhamachi, Yodogawa-ku, Osaka 532, Japan

Recombinant human NGF (rhNGF) was obtained by expression of human NGF gene in CHO cells and purified to near homogeneity from the culture medium. The neuronal cells were dissociated from 17-day fetal rat brain and seeded on a feeder layer of astroglial cells. Both rhNGF and mouse NGF (mNGF) increased acetylcholine (ACh) content and choline acetyltransferase (ChAT) activity in the neuronal cultures in a concentration-dependent manner; the ED50 was 0.5 ng/ml for rhNGF and 2.5 ng/ml for mNGF. Neurite promoting activity of rhNGF and mNGF at a concentration of 30 ng/ml was almost the same.

In rats with unilateral fimbria-formix transection, chimerogenicity (as determined by acetylcholinesterase (AChE) histochemistry in the medial septum and vertical limb of the diagonal band) were reduced to 20% of that of the controlateral nonlesioned side. Administration of rhNGF (3 or 30 µg) decrease this reduction in the number of survival cells; the survival rate was 45-55%. The effect of rhNGF in this system was less potent than that of mNGF. These results indicate that rhNGF has the same (or more potent) neurotrophic effect as mNGF in vitro and in vivo.

410.12 NEUROGROWTH FACTOR RECEPTOR EXPRESSION IN THE DEVELOPING CHICK HIPPOCAMPUS. Dario Marchetti, Lanny J. Haverkamp and James L. McMannomen. Department of Neurobiology, Houston, Texas 77030

To investigate the presence of receptors for nerve growth factor (NGF) in developing chick embryos, labeled 8-NGF was used as a probe in a biochemical assay performed on chick spinal cords from embryonic day 6 (E6) to E15. Strong NGF expression was observed as early as E6. High-affinity (K=D 19 pM) and low-affinity (K=D 10 µM) NGF. At this stage of development, we found values of NGF/beta-cell equal to 4,000 and 44,000 for high- and low-affinity NGF, respectively. These values declined with marked differences in the amount of these receptor sites throughout the developmental time course; by E15 no specific NGF binding sites could be detected.

Interestingly, the temporary distribution of NGF receptor varied according to the type of NGF considered; high-affinity NGF virtually disappeared by E10 (80% present compared to E5 value) while at the same developmental stage we were able to detect 73% of low-affinity NGF.

The transient expression of the two NGF and their differential distribution at the time of motoneuron synapse formation and cell death may shed light on NGF involvement in the development of non-classical targets for NGF action. Experiments to warrant a detailed analysis of NGF role in neuronal differentiation are in progress.


This study examines NGF modulation of signal transduction in the hippocampus (HPC) following neurotoxic lesions with colchicine (COL). Male Fischer-344 rats received COL (2.5 µg/site) or vehicle into the dorsal HPC. Immediately after COL treatment, chronic cannulae were implanted bilaterally into the lateral ventricles and perfused A1zot mini-osmotic pumps (0.25 µl/hr) with 8-NGF (10 µg/ml in ACSF) or cytochrome C (20 µg/ml in ACSF) were attached. The post-treatment animals were tested in activity chambers for 60 min. NGF treatment reduced COL-induced hyperactivity. Rats were sacrificed at 3 or 12 wks post-treatment for biochemical and histological analysis. Agents-stimulated phosphokinase (PK) turnover was assessed with [32P]-inositol was incorporated into total phospholipids. Various treatments did not alter carbachol (CAR)-induced PK turnover at 3 wks. However, NE-induced PK turnover was increased in COL/ACSFRhNGF, but not in COL/ACSFS serum samples. rhNGF stimulation of PK turnover was increased in COL-teased rats. This lesion effect was blocked when rats received rhNGF. Sections levels in the transplanted were immunocytochemically examined for CAR. COL treatment caused an increase in CAR staining in the CA3 of HPC but NGF treatment had no effect.
410.15
Molecular cloning of PC1, a novel early-inducible gene by nerve growth factor in PC12 cells. Felice Tironi, Roberta Pontielli and Andrew Bradbury.

410.16
Correlation of nerve growth factor receptor distribution and diffusibility with responsiveness and ligand-binding affinity. A.H. Rose.

410.17
The role of cytoskeletal organelles in regulating the surface distribution of nerve growth factor receptors. P. L. Spectar and J. Riding.

410.18

411.1
Induction of morphological and physiological differentiation of N1E-115 cells in serum free medium. P. Cobbet, C. Cosgrove* and A. Tijet*.

411.2
Expression of neuronal phenotype in bovine adrenal medullary chromaffin cells. S. Sleight and M.D. Browning.

Nerve growth factors VIII

Society for Neuroscience Abstracts, Volume 16, 1990
MOLECULAR CLONING OF AN NGF-INDUCIBLE KINASE: THE DEFINITION OF A NEW FAMILY OF KINASES WHICH MEDIATE RESPONSES TO EXTRACELLULAR SIGNALS. G.D. Yancopoulos, T.G. Boulton* and M.H. Cobb.* Regeneron Pharmaceuticals, Inc. 777 Old Saw Mill River Road, Tarrytown, NY 10591.

A serine/threonine kinase (MAP-2 kinase) which can phosphorylate microtubule-associated protein (MAP-2) in vitro actually acts as an intermediate in phosphorylation cascades induced by a variety of growth factors such as insulin and EGF. Evidence suggests that this MAP-2 kinase is rapidly activated following tyrosine phosphorylation by receptor or non-receptor tyrosine kinases. Activation of MAP-2 kinase is also one of the earliest detectable cellular events induced in PC12 cells by nerve growth factor (NGF). To facilitate further studies of MAP-2 kinase, we have molecularly cloned and sequenced MAP-2 kinase from a rat brain cDNA library; our cloning strategy led to the identification of additional kinases, also expressed in the rat brain, which are highly related to MAP-2 kinase. These kinases form a new family of mammalian kinases which may play crucial roles in the response to mitogens and differentiation factors. Interestingly, these mammalian kinases are most closely related to two protein kinases, recently cloned from avian and sea urchin, which are thought to play antagonistic roles in pheromone-induced cell-cycle arrest.


The diverse effects of CNTF on neuronal and non-neuronal cells, observed in vitro, suggest important roles for this factor in the development and regeneration of the nervous system. To explore its potential, we have cloned, sequenced, and expressed in E. coli the human CNTF gene. The gene codes for a 200 amino acid protein, which shares about 80% identity with its rat and rabbit homologues, and also lacks an apparent secretion signal sequence. In the optimal bacterial expression systems, recombinant human CNTF constituted approximately 30% of total cellular protein. The protein has been purified to apparent homogeneity. In dissociated, neuron-enriched cultures of E8 chick embryo ciliary ganglion, neuronal survival was detectable at 100 ng/ml; maximal activity was observed at 1-2 ng/ml, with EC50=500 pg/ml CNTF.

EFFECTS OF BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) ON RAT SEPAL CHOLINERGIC NEURONS IN CULTURE. R.F. Alderson, A.L. Alterman, *Y.A. Barde*, and R.M. Lindsay. Neurobiology Section, Regeneron Pharmaceuticals Inc., 777 Old Saw Mill River Rd., Tarrytown, NY 10591. 1. Max-Planck Inst. for Psychiatry, Dept. of Neurochemistry, D-80338 Martinsried Munich, Fed. Rep. of Germany. BDNF and NGF share extensive sequence homology (Leibrock et. al., Nature, 1989). When tested for cholinotrophic activity on E17 rat septal cholinergic neurons in culture, BDNF produced a dose dependent increase in CAT activity. The maximum response occurred with 50ng/ml of BDNF and produced a 2.2-fold increase in enzyme activity. Time course studies demonstrated a linear rise in CAT activity during the first 3 to 6 days in vitro at which time the change plateaued. The extent of the increase in CAT activity produced by BDNF was equal in glia-containing or neuron-enriched cultures suggesting that BDNF is not acting through glia. The accumulation of choline via a Na+-dependent choline-uptake mechanism was increased 3.8-fold following treatment with 50 ng/ml of BDNF. The number of acetylcholinesterase positive cells per well was also increased, 2.5-fold, as compared to the non-treated controls following a 12 day treatment with BDNF.


The restricted neuronal specificity of NGF has long suggested that other neurotrophic factors exist which act on neuronal populations that are refractory to NGF. Recent cloning of brain-derived neurotrophic factor (BDNF) has revealed that it shares close structural similarity to NGF. Using a PCR-based approach that exploited this similarity, we have identified a novel member of the NGF-BDNF gene family, termed neurotrophin-3 (NT-3). Like NGF and BDNF, NT-3 is synthesized as a precursor protein that is cleaved to form a mature protein that has 57% and 58% amino acid sequence identity with NGF and BDNF, respectively. Recombinant NT-3 protein displays distinct neuronal specificity, compared to NGF and BDNF, when assayed on elongated and dissociated cultures of PNS and CNS neurons. In contrast to the limited peripheral distribution of BDNF mRNA, NT-3 and NGF transcripts are widely expressed among adult rat peripheral tissues. In the adult CNS, all three factors are expressed at highest levels in the hippocampus; NT-3 and BDNF share other similarities in their CNS distribution. Interestingly, particular cell populations of fetal and newborn rat express NT-3 at dramatically higher levels than NGF and BDNF. The specific detection of localized, abundant NT-3 transcripts early in development points to populations of neuralblasts/neurons that may be dependent on NT-3 for their proliferation, differentiation and/or survival.


Molecular cloning and sequence analyses of the nerve growth factor (NGF)-related neurotrophic factors, brain-derived neurotrophic factor (BDNF) and the recently identified neurotrophin-3 (NT-3), have revealed striking conservation among a variety of species. All of these factors appear to be synthesized as larger precursors that are cleaved to release the mature proteins which can support the survival of distinct neuronal populations. Analyses of the precursor sequences reveal homologous regions which may be important in precursor function. Comparisons between the mature protein sequences highlights regions that may be important in determining the distinct neuronal specificities of these factors. We have functionally expressed a series of genetically-modified NGF, BDNF and NT-3 molecules. The biological activities of these modified neurotrophic molecules provide unique insights into the relationship between their structure and function.

The human CNTF gene has been identified and cloned, and its protein product expressed at high levels in E. coli. Monoclonal antibodies were obtained by fusion of splenocytes from mice immunized with recombinant and recombinant protein to SP2/0 myeloma cells. Positive hybridomas were identified by an antibody-capture assay using CNTF adsorbed to polystyrene.

One set of antibodies derived from a single immunized mouse react strongly with recombinant human CNTF but not with recombinant rat CNTF in the antibody-capture assay and by Western immunoblotting. None of these antibodies binds to a truncated CNTF protein lacking the 16 carboxy-terminal amino acids, obtained by deleting sequences downstream from a unique BamHI restriction site in the human gene. This truncated CNTF retains potent neurotrophic activity. Thus, the antibodies recognize human-specific epitopes in a non-essential region at the carboxy-terminus of CNTF. Several immunosuppression of human tumor-derived cell lines were found to have specific binding sites for CNTF and to respond to the factor, as judged by the induction of c-fos mRNA. The monoclonal antibodies to the carboxy-terminus of human CNTF may be used to detect the ligand when bound to such cells. These antibodies may therefore prove useful in the isolation and characterization of CNTF receptors.


Developing embryonic neurons require a target-derived trophic factor for survival. A protein distinct from other known nerve-inducing proteins was partially purified from muscle tissue (Oh et al., Dev. Biol., 127:188, 1988). A CNTF preparation from 6-day-old chick embryos were used in assessing the trophic activity of this protein. Approximately 25% of the neurons initially seeded survived for 6 days in the presence of the protein. The survival rate was further enhanced by the addition of laminin (Oh et al). However, the specific activity of chicken acetylcholine esterase and the level of activity of the high affinity choline uptake system were not changed after 6 days. This suggests that although this trophic factor supports motor neuron survival, it has no specific effect on the cholinergic properties of motor neurons. The protein was also found to support the survival of chicken embryonic dorsal root ganglionic neurons and sympathetic ganglionic neurons. The survival of both DRG neurons and sympathetic neurons was increased further by the presence of NGF. The trophic protein has been purified and migrates as a doublet with Mw of 53 and 57 kDa on SDS-gel electrophoresis. Preliminary amino acid data suggest that the 53 and 57 kDa proteins are either closely related or identical. (Supported by NIH grnt NE 15013 and by the Breslauer Research Fund).

411.13 BASIC FIBROBLAST GROWTH FACTOR ENHANCES THE NGF RECEPTOR PROMOTER ACTIVITY IN HUMAN NEUROBLASTOMA CELLS: CHP100, M. Tuul, T. Tai*, L. L. Decker* & M. Bothwell, Dept. of Physiology & Biophysics, Univ. of Washington, Seattle, WA 98195.

Human neuroblastoma cells (CHP100) have been used to develop an in vitro model for sympathetic neuron differentiation. Basic fibroblast growth factor (bFGF) induces morphological changes in CHP100 cells, including neurite outgrowth and neurite retraction. NGF does not induce any morphological changes in CHP100 cells, although immunocytochemical staining shows an endogenous expression of the NGF receptor (bFGF). To assess the effect of NGF and bFGF on the NGFR promoter, whose activity is known to be developmentally regulated, a 6.7 kb 5'-flanking region containing the NGFR promoter and isolated from the hGRG genomic clone and linked to the reporter gene chloramphenicol acetyltransferase (CAT). This hGRG promoter-CAT fusion was transfected into CHP100 cells, and stably transformed cells expressing CAT activity were selected. The level of CAT activity in these cells was examined following exposure with bFGF. In CHP100 cells, bFGF increased the CAT activity in these transformed cells. However, NGF (100 ng/ml) did not significantly change the CAT activity. These results indicate that the NGFR promoter activity in CHP100 cells is initially regulated by bFGF, but not by NGF.


The effects of brain derived neurotrophic factor (BDNF) on the survival and maturation of dopaminergic neurons of the presumptive substantia nigra were examined in vitro. Neurons dissociated from the rat ventral mesencephalon were cultured under serum-free conditions. Immunocytochemical staining with antibodies to tyrosine hydroxylase (TH) and uptake of [3H]-dopamine were used to monitor the survival and maturation of dopaminergic neurons present in these cultures. In the absence of exogenous neurotrophic factor(s), there was a 67% loss of TH positive neurons between day three and day eight in culture; although dopamine uptake showed an initial increase during the first three days in culture. A single treatment with BDNF one day after placing the cells in culture increased the survival of TH positive neurons by 1.9-fold eight days later. The relative increase observed by culture day 11 was 2.7-fold. The response to brain derived neurotrophic factor was dose-dependent and specific, in that nerve growth factor (NGF) was without effect. Experiments in which BDNF were delayed for several days indicated that observed effects of BDNF were mediated by increasing cell survival and not merely by increasing expression of TH immunoreactivity. This suggests future implications for the development of a novel therapeutic approach to Parkinson's disease.

411.15 REGULATION OF NERVE GROWTH FACTOR SYNTHESIS BY FIBROBLAST GROWTH FACTOR IN ASTROCYTES K. Yoshida & F. H. Gage, DEPT. OF NEUROSCIENCES, UNIVERSITY OF CALIFORNIA-SAN DIEGO, LA JOLLA, CA 92037.

The mechanism regulating NGF synthesis in astrocytes was investigated. Astrocytes obtained from newborn rat brains were seeded on a 24-well culture plate. NGF levels were measured by RIA using two-site enzyme-immunoassay. NGF secretion by astrocytes was highest just after passage, and then gradually decreased. There was no difference in NGF secretion by astrocytes from five regions: cerebral cortex, striatum, hippocampus, septum, and cerebellum. The effects of growth factors and lymphokines were tested. Although PDGF and NGF had no significant effect, each of acidic and basic FGFs, EGF, IL-1, and TNF-α increased NGF secretion. Among these, aFGF has the most potent effect, and the next is bFGF. IL-1, and TNF-α increased NGF secretion in the presence of aFGF, while bFGF and EGF did not. The peak of NGF secretion occurred, 3-12 hrs after the addition of aFGF. On the other hand, increase of cell number became significant 12-48 hrs after FGF-stimulation. Although astrocytes become fibrous-shaped by FGF-stimulation, no significant morphological change was observed in the first 12 hrs. NGF secretion by astrocytes in the presence or absence of aFGF was completely inhibited by the addition of cyclobeximide or actinomycin-D. FGFs also increased NGF secretion by fibroblasts derived from meninges, but not by microglia. These results indicate that FGFs activate astrocytes to produce NGF in damaged brain in concert with other growth factors and lymphokines.

411.16 DIFFERENTIAL EFFECTS OF aFGF AND bFGF ON SYMPATHETIC OUTGROWTH FROM NEONATAL MOUSE SUPERIOR CERVICAL GANGLIA IN VITRO. M. Druschmeyert, K.W. Brodie and J.N. Dudai, Neurobiology Division, Dept of Medicine; Duke University Medical Center; Durham, NC 27710.

Fibroblast growth factor appears to influence the kinetics and morphology of neuronal outgrowth in a variety of in vitro models. We have compared the effects of human recombinant acidic FGF (aFGF) and human recombinant basic FGF (bFGF) on various concentrations (10, 50, 100 and 500 ng/ml) on neurite outgrowth from sympathetic neurons. Superior cervical ganglia from 2-3 day old mice were cultured for several days on collagen-coated coverslips. The lengths of extending neurites and the outgrowth areas were measured using image analysis (Ganglia morphology was assessed with light and scanning electron microscopy. In the absence of growth factors, the outgrowth was sparse, consisting of non-neuronal cells (NNC) and few scattered neurites. Both aFGF and bFGF stimulated the asymmetrical outgrowth of sympathetic neurites in vitro. In addition, aFGF consistently promoted the outgrowth of neurites. They formed a fine and relatively dense network. The neurites grew on top of the non-neuronal cells to which they were closely associated. In contrast, outgrowth elicited by bFGF was not consistent and was seen mostly at the highest concentrations tested (100, 500 ng/ml). The morphology of this neurite outgrowth was similar, but less dense than that observed with aFGF. Heparin was required for the aFGF effects.

Thus aFGF is more potent than bFGF in stimulating sympathetic neurite outgrowth. FGFs may have different effects on sympathetic neurites or indirectly stimulate outgrowth by acting on the NNC. Supported by the NIH (NS 60533) and the Veterans Administration.
412.1
Instituto de Biofisica, UFRJ, Rio de Janeiro, 21244, Brazil.

Previous studies indicated that the survival of developing ganglion cells "in vivo" depends on other retinal cells. We are now studying the degeneration of retinal ganglion cells "in vitro". Retinas of newborn rats were retrogradely labeled with horseradish peroxidase injected in the posterior colliculus. One day later, the retinal cells were dissociated and cultured for 1-3 days, and the survival rate of labeled ganglion cells was evaluated. We tested the effects of culture media conditioned for 4-7 days on aggregates or explants of neonatal retinal cells. It was found that both conditioned media increased about twice the rate of survival of ganglion cells after 2-3 days in culture, when compared with control medium. The data presented here suggest that soluble molecules released by intrinsic retinal cells contribute to the control of ganglion cell survival "in vitro". (CNpq, FINEP, FAPERJ, CEPERJ-UFPR).

412.2
TROPHOMORPHISM: A UNIFYING HYPOTHESIS TO EXPLAIN NEURAL REARRANGEMENTS. R. A. Crane and R. N. Saffran, Dept. of Neurosurgery, University of Cincinnati College of Medicine; and R. L. Saffran, Department of Neurosurgery, University of Cincinnati, Cincinnati, OH 45202.

The results of experiments in which intraventricular infusion of NGF was found to cause remodeling of mature uninjured sympathetic axons led us to postulate that alterations in trophic support in the absence of injury may be sufficient to elicit axonal remodeling (trophomorphism) (Saffran and Crane, Brain Research, in press). A review of several other examples of developmental and injury-induced neuronal rearrangements also provide support for this hypothesis. We propose the following statements to support this hypothesis is based on the following postulates: 1) Nerve cells require a minimum amount of trophic factor for survival (trophic theory of neuronal connections), 2) Nerve cells extend neurites in order to obtain trophic support, 3) Nerve cells maximize the amount of trophic support acquired relative to the volume of cytoplasm they maintain, 4) Neuritic branches from the same neuron cell compete with each other for growth materials from the cell body (Smithelser and Crane's "sibling neuron bias" hypothesis. J. Neurobiol., 15:517, 84). If these postulates are correct, changes in the distribution of trophic support, or injury to axonal branches that disrupt the acquisition of trophic factor by the nerve cell, result in neuronal remodeling in order to maximize trophic support to the cell body. Such a mechanism is consistent with normal developmental remodeling of axonal arbors, the retention of normally-transient branches following elimination of collaterals, and injury-induced neuronal rearrangements in maturity. (Supported by NS-17131 and AG-07691).

412.3
NETWORKS FORMED BY DORSAL ROOT GANGLION CELLS IN ORGANOTYPIC CULTURES: A COMPUTER-AIDED ANALYSIS OF HPR INTRACELLULARLY LABELED NEURONS. M.C. Calvet, M.J. Brian*, and J. Calvet**, INSERM U-396, EPHE, Institut de Biologie, 94060 Montpellier, FRANCE.

The patterns of growth, the arborizations and the neuritic pathways formed by fetal rat dorsal root ganglion (DRG) cells were compared when cultured with or without attached spinal cord explants. Intracellular isotope-horseradish peroxidase staining of the cultured DRG neurons allowed a 3-dimensional reconstruction of these individual cells and a morphometric analysis of their neuritic processes. Two populations of DRG neurons were studied: 1) isolated DRG cells explanted at 15 to 17-day fetal rats. 2) DRG cells with their attached transverse cross-section of whole spinal cord and explanted at 13 to 14-day fetal rats. When compared to attached DRG neurons, the isolated DRG neurons showed longer neuritic lengths, larger neuritic field areas and more numerous branches developing in a multipolar way. The DRG neuron attached to spinal cord had significantly smaller and mostly bipolar processes with well individualized central and peripheral branches. Such preliminary results indicate that the spinal cord (and more precisely the target cells of the dorsal horn) acts as a prominent factor upon the development of the DRG neuritic networks. (Work supported by IPEN-RJ-BEAULFUR).

412.4
PRODUCTION OF DIFFERENT TRUNCATED FORMS OF RECOMBANT HUMAN NERVE GROWTH FACTOR RECEPTOR IN A BACULOVIRUS SYSTEM. Y. Prabhakar, J.L. Gorter*, and A.H. Ross, Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545.

Recombinant extracellular domain (RED) of human nerve growth factor receptor (NGF-R) is being produced in bulk quantities for biochemical characterization and X-ray crystallography using a recombinant baculovirus in Sf9 (Spodoptera frugiperda) cells in suspension culture. The RED in preliminary studies was found to be a monomer by chemical crosslinking and equilibrium centrifugation. The RED is asymmetric with a frictional coefficient of 1.7. When recombinant virus encoding RED was injected into Trichoplusia ni larvae, the RED produced (TRRED) was found to be glycosylated unlike that produced in Sf9 cells (SGRED). There does not seem to be any significant change in the binding pattern of TRRED with 125I-NGF compared to the SGRED. TRRED is heat stable retaining at least 60% of the specific binding even after heating at 100°C for 15 min. This stability may result from the large number of RED disulfide bonds. Binding studies of TR-RED with NGF-responsive but NGF-lacking neuronal cell types are in progress, in a bid to identify the NGF-R associated proteins. For the same purpose, other recombinant viruses encoding the extracellular, the transmembrane and intracellular domains are being produced.

412.5
IN VITRO ANALYSIS OF ASTROCYTE MEDIATED NEURITE BEHAVIOR IN THE HIPPOCAMPUS OF THE GRAY SHORT-TAILED OPOSSUM, E.S. Lartaud and E.A. Rollins, Department of Veterinary Anatomy, Iowa State Univ., Ames, IA 50011.

The intimate relationship between the neurons and glial cells plays a key role in the development of the central nervous system. In this study we examined changes in neurite morphology which occurred when neurons and astrocytes isolated from the hippocampi of the Brazilian gray short-tailed opossums were co-cultured under various conditions. Astrocytes were harvested on day P19 from opossum hippocampi and grown to confluency. Conditioned medium was collected every 2 days for 10-14 days. At this time the cells were trypsinized and co-cultured with hippocampal pyramidal cells subjected on day P8-9. Control cultures of hippocampal neurons alone were similarly treated. Glial monolayers were replaced 1 day prior to the seeding of hippocampal neurons. As expected, it was observed that neurons grown on glial monolayers tended to be multipolar with extremely lengthy processes. Further, their long-term survival was greatly enhanced compared to control cultures. Astrocyte-conditioned media also enhanced neurite outgrowth and survival of astrocyte-free cultures. In contrast, when neurons and astrocytes were dispersed and plated at ratios of approximately 5 to 1, neurons exhibited multipolar, bipolar, or unipolar characteristics depending on the distance between the cells and the level of neurite-neuron and neurite-astrocyte contact. If the neuron made no contact with a glial cell or if contact existed but its soma was within 3 cell bodies of the astrocyte there was a high probability that the cell would display multipolar characteristics. If the neuron was more than 3 cell bodies away and made contact with the glial cell alone, there was a high likelihood that the neuron was unipolar. Neurons which were in contact with other neurons generally remained multipolar regardless of their relationship with the astrocyte. These findings suggest that both soluble and membrane bound glial factors exert influence on neurite outgrowth during development.

412.6
CULTURED RAT ASTROCYTES EXPRESS FUNCTIONAL INTEGRINS. N.J. Twill and S. Carbone, The Centre for Neurosciences, McGill University, Montreal, General Hospital Research Institute, Montreal, Canada H3G 1A4.

We had shown earlier that Mob 3A3, which is directed against the β3 integrin subunit, labelled the cell surface of rat astrocytes (Twill et al., 1990, Biochemistry, in press). Using polyclonal antibodies against the β1, β2, β3, and β5 integrins, we used specific cell attachment assays we have shown that astrocytes bind to different extracellular matrix molecules such as laminin, collagen, fibronectin, and vitronectin through receptors of the integrin family. Immunoprecipitation of these integrins suggests that two heterodimers (α6β1 and α5β1) participate in the recognition of laminin, collagen and fibrinectin. Astrocytes also express a heterodimer from the β3 subclasse, α6β3, which mediates the attachment of astrocytes to vitronectin. We have also shown that Schwann cells and oligodendrocytes express β1 and β3 integrins. These data suggest that integrins are important for glial-matrix adhesion. We are presently looking at integrin expression and function on glia in vivo.
413.7 GANGLIOSIDE GM1 POTENTIATES THE RISE IN INTRACELLULAR FREE Ca++ DUE TO K+ DEPOLARIZATION IN PC12 CELLS. R. S. Hibbs and M. Levine. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

Gangliosides are highly enriched components of the neuronal cell surface which exert neurotogenic and neurotrophic effects when added exogenously to cells in culture. These effects have been shown to serve as nerve growth factor (NGF)-induced neurite outgrowth and NGF-mediated activation of protein kinases in PC12 cells. Ganglioside GM1 stimulates a tyrosine phosphatase-dependent phosphoprotein activity in K+ depolarized NGF treated cells (Hibbs and Levine, submitted) suggests that gangliosides may modulate signal transduction pathways dependent upon the entry of extracellular Ca++++. Ganglioside-free Ca++ could account for these effects. We tested this possibility by measuring the changes in [Ca++] due to K+ depolarization with GM1 or GABA treated PC12 cells cultured on poly-L-lysine/siamic acid coated glass coverslips were incubated in the presence or absence of 10 μM GM1 for 2 hours by incubation with 1 μM Fura-2 AM for 30 minutes. Fluorescence intensities were measured from single cells after excitation at 355 and 380nm and [Ca++] was determined by the ratio method (Brecht, J. Biol. Chem. 260:3440). Cells were depolarized by bath application of saline containing 30-60 mM KCl for 2-5 minutes. The rise in [Ca++] was compared between identically treated pairs of individual cells from GM1 treated and untreated dishes.

GM1 treatment caused an enhanced response to K+ depolarization in ~90% of the pairs, the rise in [Ca++] due to 30 mM K+ increased from 93 ± 20 nM in untreated cells to 205 ± 41 nM in GM1 treated cells. Brief application of GM1 (1.5 mM) over a wide range of concentrations had no effect on [Ca++] by itself or when applied to cells depolarized with K+.

Thus, the exposure of PC12 cells to ganglioside GM1 for 2 hours in a significant enhancement of the ensuing rise in [Ca++] due to K+ depolarization. These results suggest that the neurotogenic and neurotrophic properties of exogenous gangliosides may be due to their ability to augment changes in [Ca++] resulting from normal extracellular stimuli.


Expression of the dopaminergic phenotype in olfactory bulb (OB) jagglomullar (GJ) neuron is dependent upon functional afferent receptor cell innervation. GJ cells are known to participate in the olfactory epithelium by intracellular irritation with ZnSO4, or unilateral odor deprivation in neonates, results in a profound decrease in OB TH activity, immunoreactivity and mRNA, when assayed 20-40 days after transection and regeneration of immunoreactive cell bodies of GLA, a neurotransmitter colocalized with dopamine in OB neurons, suggests that the cells do not die but alter their phenotype. The current study was designed to further characterize the transcriptional and translational effects of OB denervation on GM1 mRNA levels and to determine whether region- or cell type-specific alteration might occur in GAD expression. CD-1 mice were intrasylarly irradiated with 150 μG of 10% ZnSO4 (unilateral), or saline; controls (2 weeks later). Neonatal Sprague-Dawley rats were subjected to unilateral naris catarization and sacrificed 40 days after treatment. OBs were processed for TH and GAD in situ hybridization, Northern blot analysis, or immunocytochemistry. Randomly-labelled fragments of rat TH cDNA or mouse GAD cDNA (provided by G. Creelman and G. Szabo) were used as probes. By both Northern and in situ analyses, JG TH mRNA levels were greatly reduced in both ZnSO4-deaferrented @ 4% of control) and odor-deprived (56% of control) bulb, whereas JG GAD mRNA remained essentially unchanged in both rodent models. Since GAD is found in nearly all dopaminergic OB cells, the preservation of GAD message implies a differential regulation by olfactory receptor cells of TH and GAD gene expression. Supported by grants #DC0036 and MH14403.


Ascorbic acid (Asc) is the active component of a fetal brain extract that induces increased acetylcholine receptor (AChR) expression in the explants of the L5 clone of chicken muscle cell line (Knaack, Shen, Podleski & Salpeter, J. Cell Biol. 102:795, 1986). The induction of AChR expression, as characterized by binding of [125I]-labeled bungarotoxin, occurs with a delay of 20 hr (Horovitz, Knaack, Podleski & Salpeter, J. Cell Biol. 108:1823, 1989). Furthermore, we found that the delayed increase in AChRs can be triggered by a 1 hour exposure to Asc (20), time course studies suggest that an early (20 min) and intermediate compound(s) may be involved.

As has also been found to increase acetylcholine receptors in muscle cell cultures (Kalichri, Vogel, Duskin, PNAS 59:7977, 1972). We investigated regulation of collagen by Asc and asked whether collagen could be an intermediate in the Asc induced upregulation of surface AChR in the L5 cell. We found that collagen increases in response to Asc (2 fold by 6 hours), well in advance of the increase in surface AChR expression. When pepsin digested media from cultures labeled with [14C]-proline were assayed on SDS-PAGE, we identified type I, III, and V collagens as the soluble species that increase in response to Asc. We and others have shown that the monomerization of these collagens is a factor of the collagen in normal cultures. Therefore if collagen mediates the AChR response, that mediation would need to be via collagen secreted into the culture medium. When ultracentrifugal collagenase was added to cultures together with Asc, secreted soluble collagen were eliminated from the culture medium but surface receptors were still elevated to the same extent as produced by Asc alone. From these results, we conclude that Asc increases collagen secretion and surface AChR expression independently. Secreted collagen is therefore not an intermediate in the increased expression of AChR.

Supported by NIH Grants GM04524 and AR020739.

413.10 VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) CAUSES INTRACELLULAR CALCIUM OSCILLATIONS IN ASTROCYTES. J. T. Russell, A. Fatatis, P.G. Nelson, and D. F. Breenman. LDN, NICHD, Bethesda, MD 20892, and Instituto de Farmacologia, Il PoliClinico, Naples, Italy.

Previous studies have shown that low concentrations of VIP release neurotrophic substances from astrocytes in culture. However, Neurones, Res. 45: 386, 1990). We have examined the effect of similarly low concentrations of VIP on cysteic free calcium concentration in astrocytes from new-born rats in culture maintained with the fluorescent calcium indicator, fura-2 using high resolution video microscopy. The cultures were made up primarily of type 1 cortical astrocytes. Intracellular calcium ion concentration rapidly rises when fura-2 binds calcium, and the rise is followed by prolonged oscillations in Ca++ in some cells. In others the rapid rise is followed by steady, elevated intracellular calcium levels. We could not observe calcium oscillations of constant frequency (peak to peak 1 ± 1 sec) and amplitude. Upon removal of agonist the Ca++ levels ceased to oscillate and returned to resting levels. In calcium free EGTA containing (1 mM) Ringer solutions, although the initial rise was smaller, the oscillations still persisted suggesting that they are caused by release of calcium from intracellular stores. This elevation of intracellular free calcium may participate in the mechanism by which VIP receptor activation causes secretion of factors necessary for neuronal survival from astrocytes.


Previous work by many laboratories has demonstrated that N2-0-di-butyryl cyclic AMP (Bt-cAMP) can produce a differentiation of PC12 cells similar to that caused by treatment with nerve growth factor (NGF); cell division slows, neurites are elongated, and the activity of the enzymes acetyl transferase (CAT) activity is increased. The present study examines the role of N2-0-di-butyryl cyclic AMP in the actions of Bt-cAMP on PC12 cells. Treatment of PC12 cells for 7 days with n-butyrlic acid (320 μM-3.2 mM) produced a modest outgrowth of short, thick neurites and an induction of CAT activity similar to that produced by NGF, Bt-cAMP combination. In contrast, Bt-cAMP produced a similar growth of short neurites and a comparable increase in CAT. In addition, cotreatment with Bt-cAMP and dbcAMP produced a much greater than additive increase in CAT as well as potentiated outgrowth. In contrast, treatment of cultures with B- bromo-cyclic AMP produced only a modest increase in CAT activity and a greatly reduced potential of NGF. These results suggest that the butyrate moiety of Bt-cAMP contributes to the actions previously attributed to cyclic AMP.
412.13


The PC12 cell line, a rat pheochromocytoma, responds to nerve growth factor (NGF) by differentiating into sympathetic neuron-like cells. NGF or NGF-like agents cause neurites which are extended and expression of choline acetyltransferase (ChAT) is increased in response to NGF exposure. This provides an in vitro model system for the detection of NGF-mimic or -modulatory activity. In order to determine the specificity of these responses, the effects of agents with known mechanisms of action were examined. PC12 cells were treated with either test compounds alone to see if they possess NGF-mimetic properties or in combination with NGF to look for modulatory actions. After seven days, a qualitative assessment of neurite outgrowth was made and cultures were then stained and screened under light microscopy. Neurites that were produced in the presence of NGF, or NGF and the specific NGF receptor (FGF), either alone or in combination with NGF, were also examined. Cultures were examined using phase-contrast microscopy and quantitated by light microscopy. Each culture was examined by one of the authors.

412.15


We believe that microglial cells originating in nutritionally deprived astrogial cultures are counterparts of resident microglia in the CNS (Hao et al., Int. J. Neurosci. In press). Such microglia contain proto-oncogenes such as c-fms, which codes for colony-stimulating factor-1 (CSF-1) receptor. Under normal culture conditions, microglia do not release CSF-1 into cultures released between 100-300 units/ml of CSF-1 but do express CSF-1 receptor. We have also found that presence of bacterial cell wall lipopolysaccharide (LPS), microglia produce CSF-1 and tumor necrosis factor (TNF). Neither LPS nor IFN-α appreciably stimulate the secretion of CSF-1 in astrogial cultures. We found that TNF is antagonistic to the CSF-1 effect on microglia. This effect is quite different from that observed in macrophages.

We propose that under normal culture conditions microglia depend for survival and maturation on CSF-1 as a paracrine signal by the resident microglia. In the presence of LPS or IFN-α microglia become independent of astrogial by developing lipoxygenase arachidonic acid production, which is regulated by the presence of IFN-α or IFN-γ.

This work was supported by the Canadian Grant MT 4235 (S.F.1.) and a grant from the NCI (Canada) (L.J.G.).

412.16


We have previously identified a neuron survival factor that is able to promote the survival of DGN neurons following visual cortex lesions. The factor is contained in a macromolecular fraction of medium conditioned by explants of the embryonic primordia of the geniculocortical pathway and is concentration specific for two distinct populations of DGN neurons (defined by time of origin). After removal of thalamo-rubral factors, earlier generated neurons (labeled with 3H-thymidine on E14) require a 200-fold lower concentration of the factor for maximal survival compared to later generated neurons (labeled on E15/16). The distinct peaks of activity for these two populations of neurons in the neonate is no longer observed in the adult. Both E14 and E15/16 neurons could be rescued over the entire 200-fold range of concentrations tested in the infant. Thus, the neurotrophic factor retrieved from these co-cultures is effective for both populations of DGN neurons over broader and overlapping concentrations in the adult, compared to restricted and non-overlapping concentrations in the neonate. The results may reflect the strict neurotrophic requirements of young neurons during critical developmental periods. Supported by NS 16487 from NIH, NSDCS.

412.17

**BASIC FIBROBLAST GROWTH FACTOR (BFGF) PROMOTES ENTORHINAL LAYER II CELL SURVIVAL AFTER PERFORANT PATH AXOTOMY.** B.J. Cunninghams and C.W. Colman. Department of Psychobiology, University of California, Irvine, CA 92717.

The entorhinal cortex is the primary source of afferents to the hippocampus and is a focus of degeneration in Alzheimer’s Disease. In an animal model of entorhinal cell loss, a reduction of medial entorhinal cortical fibers projecting to the dentate gyrus of the hippocampal formation via the perforant path leads to selective retrograde cell loss in layer II of the entorhinal cortex. We have previously demonstrated that ventricular infusion of basic fibroblast growth factor (BFGF) spares fimbria-fornix axotomized septal cholinergic neurons from atrophy. In the present study, we examined whether injection of BFGF into the lateral ventricle of adult rats, or via cannulae implanted in layer II of medial entorhinal cortex relative to the contralateral side. In addition, many weakly stained, achromatic cells were also observed on the transected side. In contrast, no detectable changes in the morphology of layer II cells were found in the control animals. The lack of BFGF infusion could spare layer II entorhinal stellate cells. Fourteen days after unilateral transection of the perforant path, a 25% loss of Nissl stained cells was detected on the transected side. The animals were treated on the day of the operation by intracerebroventricular infusion of 5 mg/ml BFGF at 5 μl/hour over 14 days via an Alzet miniosmotic pump infused into the lateral ventricle of the rat. The mean body weight of the animals at the time of surgery was 220 ± 50 g. In addition, the number of achromatic, weakly stained cells was also reduced. Thus, ventricular infusion of BFGF can prevent cell loss associated with retrograde degeneration following axotomy to the perforant path. These findings could lead to a better understanding of the utilization of trophic factors in neurodegenerative disease.

Supported by the American Health Assistance grant to C.W.C.

412.18

**RETARDATION OF SYMPATHETIC NEURONAL OUTGROWTH IN CULTURE BY 8-AMINO ISOLOID FROM ALZHEIMER’S DISEASE BRAINS.** A.E. Bohrer*, M.J. Ball, B.S. Haves, T.D. Wadak* and Arun P. Bhatia*. Dept. of Anatomy/Cell Biology and Pharmacology, Wayne State University, Detroit, MI, Dept. of Pathology, University of Western Ontario, London, Canada.

We have examined the effects of 8-aminoisooxolo[4,5-b]quinoline (8A) on neurite outgrowth from patients with Alzheimer’s disease on the survival and neurite outgrowth of peripheral sympathetic neurons (SN) of chick embryo anterior neural crest. Core proteins (NPCP) were isolated, solubilized in 3% formic acid, precipitated by dialysis against guanidine-NCI and fractionated by Superose 12 gel filtration HPLC. The SA containing fraction was dialyzed against water. Dissociated SN were cultured in F12 medium supplemented with insulin and 10,000 U/ml of HEPES. The SN core proteins were isolated, solubilized in 30 μg/ml isolated, solubilized in 30 μg/ml 8A (30 μg/ml) caused dose-dependent reduction in the survival of both types of SN in 24 h. Higher concentrations (50 μg/ml) killed SN, whereas lower concentrations (20 μg/ml) arrested the neurite outgrowth. After washout of lower concentrations of 8A, SN recovered normal outgrowth. When SN were cultured for 2 days and then exposed to 30 μg/ml, there was gradual loss of neurites followed by cell death. However, 8A (50 μg/ml) arrested the growth of sympathetic neurons. (Supported by NIH MH 18661, AG 03047 and Atkinson Foundation.)
412.19

ACETYLCHOLINE RECEPTOR-INDUCING ACTIVITY DOES NOT INCREASE INTRACELLULAR cAMP IN CHICK MUSCLES. P.A. Johnson, D.L. Fatig, G.O. Fishbach. Dept of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO. 63110.

ARA, ACHE-Inducing Activity, is a 42kd glycoprotein purified from chicken brain that increases the synthesis of ACHRs in chick muscles, and may play a role in the development of the neuromuscular junction. We are interested in second messengers that might mediate this effect. Drugs that increase intracellular cAMP also increase the synthesis of ACHRs. Therefore ARA may act through increasing cAMP. Forskolin-free cultures of chick myotubes treated with ARA, CGFPP, a small peptide that increases the number of ACHRs and cAMP levels (Fontaine et al. J. Cell Bio. 105:1397-1402, 1989), or Forskolin, an activator of adenylate cyclase. The cells were harvested at various times from 5 mins to 24 hrs after drug treatment by measuring cAMP by a radioimmunassy sensitive to 2 fmol. Forskolin, at 100 uM, increased cAMP levels 17-fold, reaching the half maximal effect before 5 mins. CGFPP increased cAMP 3.2 fold over a similar time course. ARA produced no detectable increase. In parallel plates used to assay ACH insertion rate, measured by I25-burgatelor ton binding, ARA increased receptor incorporation, 4.2 fold, and CGFPP increased it by 10%. Furthermore, in separate experiments, the effects of ARA and Forskolin, and ARA and CGFPP were additive. As ARA had as large effect on receptor incorporation as Forskolin and larger than CGFPP and caused no detectable increase in cAMP levels, we conclude that ARA does not act via a cAMP-dependent mechanism.

412.21

ACTIVITY REGULATES EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX CODING MOLECULES IN RAT SKELETAL MUSCLE. K. Gunderson and J. Mahle. Inst. of Neurophysiology and Physics, Univ. of Oslo, N-0162 Oslo 1, Norway.

Molecules coded by the Major Histocompatibility Complex (MHC) play a prime role in immunological recognition. In normal nerve and muscle tissue these molecules are expressed at very low levels, but are up-regulated during local immunological reactions when lymphocytes invade the tissue and release certain MHC-inducing cytokines. Recently expression of MHC molecules was described in denervated skeletal muscle (Mahle, J. Brain Res. 481:368, 1989). We have observed that this phenomenon also occur in athymic animals suggesting that lymphocytes are not involved. Now we wish to ask whether the nerve-evoked action potential activity in skeletal muscle may serve as a regulator of MHC-expression.

MHC molecules were immunostained on cryosections from soleus muscles after 5-14 days of either I) retrodotoxin impulse blockade of the sciatic nerve or ii) direct electrical stimulation of denervated muscles. Inactivity was similar to denervation in causing MHC class I expression along the muscle fiber surface, and in the sarcoplasm, and in causing MHC class II expression on cells located in the interstitium of the muscle. Moreover, the MHC up-regulation in denervated muscles was strongly reduced by electrical stimulation.

In conclusion, our data show that MHC class I expression is suppressed by evoked action potential activity in normal muscle cells, and suggest that inactive muscle cells produces a signal that leads to appearance of MHC class II positive cells in the interstitium.

OTHER TROPHIC AGENTS II

413.1


Previously this laborotory reported that bovine chromaffin cells have insulin-like growth factor I (IGF-I) receptors and that these receptors are protein tyrosine kinases (Dahele et al., J. Neurochem. 53, 1989). To characterize the endogenous substrates for this kinase we have isolated phosphotyrosine-containing proteins from bovine chromaffin cells and have identified one of them as an IGF-I activated microtubule associated protein (MAP-2) kinase. Phosphotyrosine-containing proteins were isolated from control and IGF-I treated cells by adsorption to and specific elution from antiphosphotyrosine-Sephacore beads. When eluates were assayed for MAP-2 kinase activity, increased activity was found in eluates from IGF-I treated cells. The activation of MAP-2 kinase by IGF-I was varied with the time of treatment (maximal at 10-15 min) and concentration of IGF-I (maximal at 10 nm) and was potentiated by the tyrosine phosphatase inhibitor Na4VO4. Hyelin basic protein was also a good substrate for this kinase, but histones H1, H2A and H2B and ribosomal S6 protein were not phosphorylated. Treatment of the MAP-2 kinase was able to abolish the kinase activity indicating that phosphorylation of the MAP-2 kinase is required for its activity. The IGF-I stimulated MAP-2 kinase described here is similar to the insulin-stimulated MAP-2 kinase described by Ray and Sturgill (J. Biol. Chem. 261:3987, 1986) and IGF-I activated kinase cascade similar to that proposed for transduction of signals from the insulin receptor (Supported by NIH grants HL 29025 and HD 45833 and NSF grant BNS-820342).

413.2

EFFECT OF CALCITONIN GENE-RELATED PEPTIDE ON NEURITE GROWTH OF CENTRAL NEURONS IN CULTURE. Pu-Zhong Wang, Atsushi Miyazaki, Lin Chen and Xingqiu Xie. Dept. of Neurobiology, Institute of Basic Medical Sciences, Beijing 100085, China.

Effect of calcitonin gene-related peptide on neurite growth were studied in primary dissociated spinal cord cell cultures from 12-14 day mouse embryos and hippocampal neurons from newborn rat. Dissociated cell cultures were made at density of 5x10^5 cell/ml in the culture medium containing 5% NEM and 5% horse serum added nutrient supplement. O.DGR:100 mg/ml were added to culture dish containing 2ml cell suspensions. The neurite outgrowth was assessed at 2 hr. Both spinal cord cells and hippocampal neurons were grown in the presence of GFRP, the numbers and length of neurite outgrowth were markedly increased twice as much as that seen in the control cultures (p<0.01). From long-term cultures, the cell size and the number of neurites were, on the average, higher than those observed in the parallel series grown with non-GFRP medium. To quantitate the effects of the neurite growth the total content of cellular protein were measured by fluorometry. For cytomter analysis of cellular protein, control cells and GFRP-treated neurons by 12 days and 15 days were stained with fluorescein isothiocyanate (FITC) and fluorescence was measured on a single cell basis. From cellular fluorescence histograms, it was shown that the relative content of cellular protein was elevated in presence of GFRP. These results show that GFRP dramatically enhance the neural outgrowth and is involved in protein synthesis in cells.
413.3 EFFECT OF HEPARIN BINDING GROWTH FACTORS ON THE LA-N-1 NEUROTROPHIC CELL LINE E. S. S. Ottigliari, T. Maciej, R. F. Tomeski, X. Li, C. P. Matrion, and M. D. P. Neter. University of Rochester School of Medicine, Department of Neurobiology and Anatomy, Rochester, NY.

Heparin binding growth factors (HBGF) are a class of related polypeptides which include acidic fibroblast growth factor (aFGF) and basic FGF (bFGF also HBGF-2). These factors have varied effects in different tissues, including neural tissue. We evaluated the effects of various concentrations of aFGF and bFGF on the LA-N-1 neuron-like cells. Although, both factors stimulate the cells to differentiate morphologically, as seen by the extension of neurites.

Other tested forms of HBGF: HBGF-1a, aFGF with residues 1-20 inserted between residues I2 and I3, has been used in this study. We also tested similar HBGF-1 and HBGF-1a did not induce neurite formation of the HBGF-1U cells. It has been suggested that aFGF increases the amount of intermediate filaments in a cell while NGF increases the microtubule content. To examine this possibility that occurs in LA-N-1 cells, cultures were stained for neurofilament protein and tubulin. We were unable to distinguish a clear difference in staining pattern between aFGF or NGF treated cultures. We are currently examining these cultures by electron microscopy to gain a more definitive answer since microtubules and intermediate filaments are distinguishable at the EM level.

Supported by NIH NS 25778 and Predoctoral fellowship NIMH 09851

413.4 STIMULATION OF PROLIFERATION OF C6 GLIOMA CELLS BY S100B: EVIDENCE FOR A CENTRAL NERVOUS SYSTEM GROWTH FACTOR. S. H. Berger, R. H. Stelzner, and L. J. Van Eldik. Department of Cell Biology and Pharmacology, Vanderbilt University, Nashville TN 37232.

S100B is a member of a family of homologous calcium binding proteins, and the protein promotes the survival and morphological differentiation of embryonic cortical neurons. S100B production in brain is predominantly in glial cells, including the rat C6 glioma cell line. S100B synthesizes and secretes the protein. Specific reductions in S100B in C6 cells by antisense oligonucleotides resulted in a reduction in cell number in a dose-dependent manner. Exogenous S100B also stimulated [3H]thymidine labeling of nuclei and the expression of proproteins. This stimulation of proliferation by S100B complemented the effects of PDGF but not insulin. S100B was unable to stimulate the proliferation of two neuroblastoma cell lines. These data suggest the possibility that S100B acts in an autocrine manner to coordinate the growth of glial cells while the differentiation of neurons in the developing brain.

(Supported by American Paralysis Association and Cystic Fibrosis Foundation.)

413.5 PURIFICATION AND CHARTERIZATION OF RECOMBINANT BOVINE BASIC FIBROBLAST GROWTH FACTOR FROM ESCHERICHIA COLI. T. W., G. W. and T. W. ., Department of Molecular Neurobiology, China National Academy of Sciences, Beijing, China 100077, China.

The cDNA sequence coding for bovine basic fibroblast growth factor (bFGF) has been cloned into an expression vector (pBR329) downstream of the lac Z promoter. The DNA sequencing results have shown that the bFGF cDNA is inserted in the correct reading frame of the promoter. The expression of the fusion gene in Escherichia coli and pigment epithelial cells resulted in the accumulation of about 15 mg recombinant bFGF per liter bacteria culture. The recombinant bFGF purified with about 95% has been obtained by passing the bacterial lysate through an ion exchange column and a heparin affinity chromatography. The pure bFGF obtained thus far can be used to biological activities indistinguishable from the natural bFGF purified by us from bovine placenta, either in mitogenesis or in bone angiogenesis assays. Animal experiments have been shown that the bFGF can promote soft tissue wound healing and may have antihypertrophic effect. Injection of the recombinant bFGF into the shaved rabbit ear not only induced new blood vessel growth, but also promoting hair growth in the region of injection, indicating that the bFGF might be of value in the stimulation of regeneration of hair.

413.6 CLOSING AND EXPRESSION OF A DNA ENCODING A NOVEL HUMAN NEUROTROPHIC FACTOR. T. Kamihara, E. Yoshimura, E. Nakahama*, and M. Kajitani. Biotechnology Center, Riken, 2-1, Takeda, Nishioji, Narita, Chiba 275, Japan.

Two structurally related neurotrophic factors, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) were reported which are believed to play an essential role in the growth, survival, and differentiation of neurons in the nervous system.

To obtain other NGF related genes, we tried to screen a human glialoma DNA library under low stringent hybridization conditions using a synthetic DNA corresponding to human NGF as a probe. A cDNA has been cloned which encodes a novel human neurotrophic factor (designated nerve growth factor-2, NGF-2) composed of 257 amino acid residues including a prepro-sequence of 135 residues and a mature region of 122 residues. The amino acid sequence of the mature NGF-2 exhibited 58% and 55% similarity with human NGF and pig BDNF, respectively. The conditioned medium of C6-7 cells transfected with an expression plasmid for the NGF-2 cDNA supported the survival of some neural crest-derived CHO cells with the root ganglia of embryonic chick. Upon Northern blotting analysis, large amounts of NGF-1 mRNA were detected in all the tissues examined except muscle and testis, the highest level of the mRNA synthesis being observed in kidney. In rat brain, NGF-2 mRNA was detected at embryonic day 17.5, and the level of the mRNA synthesis reached a maximum 1 to 2 weeks postnataally.

These results suggest that there are at least three nerve growth factor related genes, NGF, NGF-2, and NGF-3. These factors may have distinct functions in vivo, because they showed different tissue distribution and developmental expression.

413.7 INSULIN-LIKE GROWTH FACTOR II GENE EXPRESSION IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS. D. M. Martin, P. J. Yanta, and E. F. Feldman. Departments of Neurology and Human Genetics, University of Michigan Medical Center, Ann Arbor, MI 48109.

Diabetic retinopathy is the major cause of blindness in the United States. Recent observations implicate metabolically induced neovascularization in diabetic retinopathy. Insulin-like growth factors, known to stimulate endothelial cell proliferation, have been found in the vitreous and may act directly in the eye. We postulated that insulin-like growth factor II (IGF-II) is produced in the eye and may play a role in the pathogenesis of diabetic retinopathy. We reported that cultured human retinal pigment epithelial (HRPE) cells express the IGF-II gene. We are currently investigating changes in IGF-II gene expression in HRPE cells conditioned in high glucose media. A radiolabeled antisense RNA transcript was generated from the T7 promoter of the pGem-4 vector (Promega) containing 854 bp of a human IGF-II cDNA. Total cellular RNA was isolated by the guanidium thiocyanate-phenol method and separated by formaldehyde-agarose gel electrophoresis. RNA was subjected to Northern blot analysis on nylon membranes and hybridized with the radioactive probe generated from the IGF-II probe to human IGF-I template DNA was seen. Autoradiography revealed a 1.2 Kb transcript which co-migrated with a 1.2 Kb transcript present in retinal extracts. The presence of IGF-II RNA in HRPE cells suggests that this growth factor may play a role in the pathogenesis of diabetic retinopathy.

(Supported by grant NS03183 to ELF)
413.9
CHARACTERIZATION AND PARTIAL PURIFICATION OF A NOVEL NEUROTROPHIC FACTOR SECRETED FROM HUMAN FETAL TESTIS CELLS. M.S. Hayth, R.H. Soriano & D.G. Routa, CNS Disease Research, Searle R&D c/o Monsanto, St. Louis, MO 63198
Neuronal survival factors modeled after the protoctite factor NGF, are molecularly purified by the tryptase and activate the neuronal cell line Neuro-2a. We demonstrated that Neuro-2a is localized to the cell surface and that this factor does not alter the target cell. We report here, the characterization and partial purification of a novel neurotrophic factor secreted from human fetal testis cells.

413.10
ASTROGLIAL MITOGENS AFFECT SEPTAL CHOLINERGIC CELL EXPRESSION DIFFERENTIALLY. R.L. Kenigsberg and I.E. Mazzoni, Centre de Recherche Pédiatrique, Hôpital Ste-Justine, Montreal, Que. Canada H3T 1C5
It has been proposed that the regenerative capacity of CNS neurons may be affected by astroglia at a lesion site which poses physical and/or chemical barriers. We previously found that EGF, a potent glial mitogen, is upregulated to sepal cultures from fetal rat brains, produced a decrease in cholineric cell parameters, choline acetyltransferase, (CHAT) and acetylcholine (ACh) in glutamate (Dettmers et al., J. Neurochem. 52, Suppl. S192, 1989). As these changes were accompanied by a marked glial cell proliferation and no change in neuronal survival we extended these studies to determine the specificity of the EGF response. Consequently we examined the effects of a number of glial mitogens which are released following injury in the CNS on septal cultures. Although all mitogens examined produced astroglia proliferation, only EGF decreased ChAT activity while thrombin produced a significant increase in ChAT and PGF had no effects. These results suggest that although several mitogens may affect glial cell division similarly, they may control the expression of astroglia and neurons differently.

413.11
K252a, a general kinase inhibitor selectively blocks the actions of NGF in PC12 cells. Since gangliosides have been reported to modulate neuronal cell responsiveness to NGF and to regulate several protein kinases, the ability of these compounds to revert the inhibition by K252a of NGF-induced neurite outgrowth, was tested by several experiments. Gangliosides completely prevented the inhibition by K252a of NGF-induced neurite re-generation, c-fos induction and, partially, protein kinase N activation. The ganglioside protective effects were concentration-dependent and required the intact molecule. These findings raise the possibility that gangliosides might affect a specific pathway of NGF responses sensitive to inhibition by K252a.

413.12
REGULATION OF THE SURFACE DISTRIBUTION OF GM1 BY UNDERLYING CYTOSKELETAL ORGANELLES. J.H. Fentie and F.J. Polen, Department of Anatomical Sciences and Neurobiology, University of Louisville, School of Medicine, Louisville, Kentucky 40292.
Gangliosides are relatively abundant components of neuronal membranes. They play an important role in neuronal differentiation due to gangliosidoses results in aberrant neurite formation. Neurons, including the murine Neuro-2a neuroblastoma line, undergo increased neurogenesis after exposure to the ganglioside GM1. Responses to Neurotrophic Factor- dependent and NGF-independent trophic agents can be potentiated by GM1. Thus evidence is accumulating that gangliosides, especially GM1, play a role in neuronal development, although the mechanism remains unknown. In this study, we examined the surface distribution of GM1 on Neuro-2a cells at different stages of neurogenesis and development. GM1 was localized using direct fluorescence of cholera toxin B-FITC and with indirect immunofluorescence and scanning transmission electron microscopy employing antibodies to GM1. Although a uniform distribution of label was found on perikaryal and neuronal surfaces, occasional linear arrangements suggested the possibility of an underlying fibrilar organelle. Cytoskeletal probes were used to determine their nature. Taxol (1.0 uM) treatment of Neuro-2a cells produced neurites with distal GM1 positive label and stabilized microtubules (MTs). In contrast, Colcemid (0.05 ug/ml) produced neurites devoid of GM1 label and lating MTs. Cytochalasin D (2 ug/ml) reduced microfilaments but had no visible effect on GM1 surface distribution. Semi-quantitative studies, in progress, employ the direct fluorescence of cholera toxin B-FITC and cholera toxin-gold conjugates in combination with cytoskeletal altering agents. These studies demonstrate that the surface distribution of GM1 is dependent on microtubule organization. Supported by NIH grant NS24524.

413.13
NEUROTROPHIC AND METABOLIC ACTIONS OF INDIVIDUAL GANGLIOSIDES AND THEIR POTENTIATION OF TAUROINE'S EFFECT ON NEURO-2a CELLS. C.L. Lu, G. Yorks and F.J. Polen, Department of Anatomical Sciences and Neurobiology, University of Louisville, School of Medicine, Louisville, Kentucky 40292.
Gangliosides play an important regulatory role in the development of the nervous system and may modulate the actions of neurotrophic factors. However, reports of species-specific ganglioside-mediated effects are rare. To examine the neurotrophic responses elicited by individual gangliosides, we examined the neurotrophic and metabolic actions of four major ganglioside species (GM1, GD1a, GD1b, GTb) in the presence and absence of taurine on the murine Neuro-2a neuroblastoma cell line. Taurine's neurotrophic actions on Neuro-2a cells have been recently reported (Spooen, Capi, et al., Exp. Neurol. 98, 459). In this study, computer-assisted morphometry was employed to evaluate neuritic sprouting and the number of process bearing cells after 24 hr exposure to the individual gangliosides alone or in the presence of taurine. Furthermore, the activity of ornithine decarboxylase (ODC), the rate limiting enzyme in polyamine biosynthesis, was examined. Exposure of Neuro-2a cells to media containing the individual gangliosides (100 ug/ml) or taurine (1 mM) enhanced neurogenesis. ODC activity was enhanced 1.6-1.9 fold after 6 hr exposure to GD1a, GD1b, or GTb. GM1, although neurotrophic, did not enhance ODC activity. Simultaneous treatment of Neuro-2a cells with taurine and each ganglioside potentiated the neurotrophic action of taurine. Furthermore, when submaximal levels of taurine (0.1 mM) were used, GD1a, GD1b, and GTb elevated ODC levels significantly higher (3.6-4.6 fold) than taurine or the level obtained by the individual ganglioside alone. These studies demonstrate that neurogenesis and polyamine synthesis are independent and that gangliosides may play a species-specific regulatory role in neuronal development. Supported by NIH NS24524.

413.14
GM1 GANGLIOSIDE TREATMENT DOES NOT INDUCE AXONAL SPROUTING IN ADULT HAMSTER VISUAL SYSTEM. B.A. Sabel1, L. Cavicchiolo2 and A. Lenoc1. 1. Institute of Medical Psychology, University of Munich Medical School, 8000 Munich 2, Fed. Rep. Germany and (2) Fidia Research Laboratories, 35031 Abano Terme, Italy
When hamsters are given injections of the right superior colliculus (rSC) on postnatal day 1 (P1), the retinofugal fibers from their left eye cross the midline to innervate the wrong SC. Right eye removal (RE) at a time up to P14 will result in sprouting of these fibers in the left SC and treatment with exogenous gangliosides (GM1) enhances this sprouting after RE at P9 (Sabel, B.A. and Schneider, G. Exp. Brain Res. 71: 117-125, 1989). We now report the effects of GM1 in a preparation where sprouting does not occur normally (i.e. RE after P14) or when the sprouting is vigorous (RE on P1).

Hamsters received rSC lesions on P1, with RE on P1, P18 or P30. Groups of hamsters (n=7-9 each) were then treated i.p. with 30 mg/kg GM1 (Fidia Res. Labs.) or saline. Sprouting onset at RE was determined by weekly period of daily treatment, the retinofugal pathway and left SC innervation was demonstrated by removal of the left eye and staining degenerating axon terminals using the Fink-Heimer method. Analysis of the size of the terminal field in the left SC clearly revealed the previously known decline in sprouting vigor with increasing age, but GM1 was without detectable effect on the extent of sprouting in hamsters with RE at P18 or P30.

Thus, GM1 does not enhance axonal sprouting when maximal sprouting vigor is present. Also, GM1-treatment does not elicit axonal sprouting in the adult hamster visual system, suggesting that GM1 is only effective during an ongoing sprouting response (as at P9).

Supported by DFG grant SF220/10 and Fidia Research Labs.
413.15

To study recovery of function in a definable model of brain injury, we have previously employed the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced damage to the nigrostriatal dopamine (DA) system in the male C57 Bl/6 mouse (Acu Physical Sci. Adv. Med. 1993, 2, 580). MPTP induces lesions in the substantia nigra and related terminal regions of the nigrostriatal dopamine DA system, resulting in a progressive loss of dopaminergic neurons and ultimately the development of motor dysfunction (Dowedani et al., in prep.) Using this paradigm it is possible to simulate variable degrees of axonal injury from "mild" to "severe", resembling "diffuse axonal injury (DAI)", a primary response of the brain to traumatic injury in humans. We now report the effects of GM1-ganglioside treatment on functional outcome after graded crush of the rat optic nerve using two behavioral tests: Spinothalamic tract stimulation and visual field loss.

In the orientation paradigm, rats were trained to orient towards a visual stimulus in the visual field ipsilateral to the, the unilateral crush. Here, initial loss of function was followed by a brief period of recovery. Following brain crush, when rats were treated with 50 mg/kg GM1 (p), they performed, on average, better than control rats on all postoperative days, but significant differences were seen only on day 7 post crush. In the brightness-discrimination paradigm, bilaterally crushed rats had to choose between a bright or dark stimulus in a maze to obtain water reward. Although the deficit and subsequent recovery of function is similar to that of the orientation paradigm, GM1 was without detectable effect on brightness-discrimination performance.

These behavioral results can be taken to suggest that ganglioside treatment can reduce the detrimental effects of unilateral optic nerve, but a suitable and sensitive behavioral paradigm is needed to demonstrate efficacy of treatment.

413.17
EPIDEMIC GROWTH FACTOR (EGF) ENHANCES, IN RATS, DOPAMINERGIC PATHWAY "IN VIVO" AN IMMUNOHISTOCHEMICAL STUDY. *A. Zucconi, G. Pirazzoli, A. Riccio, B.E. Burke, R. Fabrici, R. Furlan, C.B. Marini, G. Scorsati,* and A. Cattaneo.* U.O. di Neurochirurgia, Ospedale Civile, Modena, Italy. 413.18 EPIDEMAL GROWTH FACTOR IS A MITOGEN AND INCREASES DOPAMINE UPTAKE IN RAT EMBRYO MESENCEPHALIC PRIMARY CULTURE. *D. Casper, C. Mytilineos, and M. Blum,* Fishberg Research Center in Neurobiology and Department of Neurology, Mount Sinai Medical Center, New York, NY 10029.

Epidermal growth factor (EGF) has previously been described as a mitogen on epithelial- and mesenchyme-derived tissue. In the central nervous system, EGF has also been shown to be a mitogen for astrocytes. Our group has previously reported that EGF can increase neuronal and glial dopamine uptake in this cell culture system (Casper et al., Soc. Neurosci. Abst., 17/509, 1989). In this study, we have placed dissociated mesencephalic cell cultures, or neuro-sphere cells, into the presence or absence of the labelled neuropeptide. These cells are seen by their large and flattened nuclei, which lightly stain with tau immunocytochemistry, or by their nucleolus and scant cytoplasm, soon by phase microscopy. A few cells stain very weakly with antibodies to either GFAP or tuft. While this population of "undifferentiated" cells increases, EGF also increases. Using immunocytochemistry for tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine biosynthesis to mark dopaminergic cells, we found that the number of cells expressing this marker decreases up to a week in culture, but the number of these cells is stable after this in EGF treated cultures up to 22 days. The number of TH-positive cells in control cultures continues to decrease. Studies to be presented will determine whether the decrease in dopamine uptake is due to the action of EGF on the existing stabilized dopaminergic neurons such as inducing neurite outgrowth, or whether it is causing differentiation or neurogenesis of new dopaminergic neurons.

413.19
EPIDEMAL GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR-ALPHA mRNA EXPRESSION IN PCD AND WEAVERTY MAMMUT ANIMALS. *L. M. Lazar, K. A. Kelsey, and M. Blum,* Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029.

We previously investigated the effects of epidermal growth factor (EGF) in the mammalian CNS have focused on the expression of specific mRNAs in specific regions. We now report on our preliminary findings regarding the changes in level of mRNA expression for EGF and its structural homolog transforming growth factor-alpha (TGF-alpha) in cerebellar and lower brainstem of Pkunja cell derived cultures of weaver mutant mice. Using a R1 PCR protection assay, we have detected elevated levels of EGF mRNA in cerebellar and severely affected ventral mesencephalon (PGF) and compared this to non-atonic sex matched littermates. TGF-alpha mRNA levels, however, detected at levels ~80 times higher than that of EGF in the wild-type (+/+) cerebellum, are not significantly altered in this mutant model. In contrast, in the cerebellum of weaver mutant mice in which the neurodegenerative process is specific to the granule population and in which the weaver mutation (Ww) displays cerebellar histopathology intermediate between mutant (ww) and heterozygous normal (Ww) animals, levels of TGF-alpha mRNA are approximately 2 and 1.25 times greater for homozygous mutant (ww) and heterozygous normal (Ww) cerebellar than levels detected in cerebellum of homozygous normal (+/+), sex matched, heterozygotic animals. Interestingly, levels of TGF-alpha mRNA in lower brainstem (Mesencephalon-Pons) of the weaver mice are (30%) that of levels in cerebellum of the weaver mutant mouse. Our findings of differential gene expression for EGF and EGF-like molecules, then, suggest that while both neurotrophines utilize a common receptor, their physiological roles in brain may be distinctly.

413.20

Exposure of mesencephalic cultures to epidermal growth factor (EGF) results in long term stimulation of dopamine DA neurogenesis. We have previously reported (1) MPP+ exposure to cultures of mesencephalic cells results in a marked decline in their survival and axon outgrowth. Mesencephalic cultures established from E-16 rats were plated at 5x10^3 cells/cm^2 in the absence or presence of 50 Micromolar MPP+ and allowed to grow for 21 days. MPP+ was then added to the appropriate groups and was present throughout the experimental period. Cultures were exposed to 10 and 50 Micromolar MPP+ for 4 hours on the 4th day in vitro (DIV). At this time, there was no significant difference between control and MPP+ treated cultures with respect to DA uptake and number of TH+ process bearing neurites. Following removal of MPP+ on DIV 4, a similar number of neurites were present. It was observed that exposure to MPP+ resulted in a decrease in the number of TH+ cells (45% of controls) and loss of TH+ cells (45% of controls) was observed in control and EGF-treated cultures, suggesting a lack of a protective effect of EGF against MPP+-induced DA neuron degeneration. The greater effect of MPP+ on DA uptake probably reflects the loss of DA neurons, combined with reduction in neurite length in the surviving TH+ neurites. DA uptake remained lower in the MPP+-treated cultures compared to their respective controls during further in vivo development, but EGF treatment resulted in a pronounced increase in uptake both in control and in MPP+-treated cultures. On DIV 19 the MPP+-treated cultures exposed to EGF had uptake levels ~10-fold greater than MPP+-treated cultures. In the control cultures, the number of cells declined on DIV 12 and 19, but this decline was not observed in EGF-treated cultures. It still remains to be determined whether all of the TH+ cells on DIV 19 are the surviving neurons which were spared from the toxic effect of MPP+. Supported by NIDH grant NS-07245, NS-23017 and the United Parkinson Foundation.
413.21

Low concentrations of adenine and guanine nucleosides and nucleotides, when added to the medium of serum-deprived quiescent astroblasts or human astrocytoma cells, stimulated cell proliferation in a dose-dependent fashion. We used agonists and antagonists specific for various types of cell-surface adenine and purine receptors to determine which receptors were involved. Adenosine had two peaks of activity, at 100 nM and at 7.5 μM. Guanosine and inosine stimulated cell proliferation more than adenine in μM concentrations, but were inactive at mM concentrations. Activation of astroblast A2 receptors both stimulated cell division and increased intracellular cAMP.

Purine nucleotides stimulated astroblast proliferation in μM concentrations. However, ω-methylpyridine A7, a preferential P2x agonist, maximally stimulated proliferation at 3 nM. ω-Methylthio ATP, a preferential P2y agonist, had peak activity at 10 μM. This implies that purine nucleotides stimulate astrocyte mitosis through a P2y receptor.

Purine nucleosides and nucleotides are released in high concentrations in the CNS during neurotransmission, hypoxia and cell death. Our data imply that this may be sufficient to stimulate proliferation of various types of adjacent cells. (Supported by a grant from the Ontario March of Dimes)

413.22

C6 rat glioma cells incubated in serum-free medium with D-[14C]glucosamine secreted upon stimulation with nerve growth factor (NGF) or D-[14C]glucosamine (6.5 μM) a number of glycoproteins (Gp) the most prominent of which is a 270, 220, and a 69 kDa Gp. Several growth factors, hormones and phospholipids are known to induce [35S]methionine release. Significant levels of the 220 kDa Gp were detected after pulse labeling with [35S]methionine in the presence of NGF, after 15 min incubation immunoprecipitable radioactively labeled J1/tensacin was noticed. Tunicamycin inhibited most profoundly release of the 220 kDa Gp labeled either by D-[14C]glucosamine or [35S]methionine.

These results extend the range of neurotrophic properties attributed to NGF to cells of gial origin and suggest that NGF regulates secretion of extracellular matrix proteins. The data also suggest that NGF stimulation of fibronectin and J1/tensacin secretion is presumably mediated by an NGF or NGF-like molecule also secreted by the C6 glioma cells.

413.23

The various types of neural crest-derived peripheral neurons can be characterized by their distinctive combinations of neurotransmitters and neuropeptides. Neuronal phenotypes respond to environmental cues in vitro and in vivo. The cholinergic neuronal differentiation factor (CDF) from cultured heart cells is identical to leukemia inhibitory factor (LIF) and is preferentially induced by heart cells. CDF can induce cholinergic differentiation in cultured rat sympathetic neurons (Yamamoto et al., Science 248, 1412, 1989). We find that CDF also induces the expression of substance P, somatostatin and vasoactive intestinal polypeptide, while reducing the expression of neuropeptide Y in a dose-dependent manner in the sympathetic neurons. The alterations in peptides are also observed at the mRNA level. The changes in peptide expression are reversed upon removal of CDF from the neurons, demonstrating that the factor is required for maintenance of the newly induced phenotypes. Dorsal root ganglion neurons also respond to LIF, but with different phenotypic changes, suggesting that CDF/LIF could have widespread effects in the nervous system.

413.24

Transplants of normal RPE cells in retinas of RCS dystrophic rats has been shown to restore photoreceptor cell (RCC) loss, even when transplanted into the RPE transplanted, suggesting RPE rescue may be affected by an RPE diffusible trophic factor(s). In one study, normal RPE cells were transplanted into the subretinal space of 18-week-old RCS dystrophic rats, separated from the RPE by Bruch’s membrane. Seven weeks later, RPE rescue was detected under and lateral to the eye but not to the transplant. In a second study, normal RPE cells were emasculated in permselective hollow filters (slow release mechanism) and placed in cultures of PMCs, isolated from day 2 Long Evans rats, and grown in a defined medium, lacking serum. Under these conditions, PMCs survived for at least a week, while in control filters, lacking RPE cells, PMC survival was not observed. In addition, PMCs were supplemented with several growth factors. Only epididymal growth factor appeared to significantly enhance survival. These studies provide strong evidence that RPE cells secrete a factor(s) which affects RPE rescue and survival. Results of the slow release mechanism and vitreous injections in RCS dystrophic rats will be reported. (Fitch for Sight, Inc. and NEI 04337)

413.25

Evidence for the existence of TGF-alpha prepro mRNA in rodent brain has been reported (J. Neuroscience 8:1901, 1988) but limited information is available on the localization of TGF-alpha in rat brain. We report TGF-alpha levels in rat brain as measured by a specific RIA developed in our laboratory. Chemically synthesized rat TGF-alpha was used for antisera generation, iodination and reference standard. The RIA is sensitive to 8 pg/tube. Whole brain, cerebrum, cortex, cerebellum, and midbrain of adult rats were homogenized in 50mM phosphate buffered saline (pH 7.4) and centrifuged at 100,000 g for 60 minutes. The supernatants were used for quantification of TGF-alpha and protein contents. TGF-alpha concentration is expressed as pg/g protein (values are mean ± SD). Whole Brain: 47±8; Cerebral Cortex: 72±16; Cerebellum: 162±18; Midbrain: 51±9. Conclusion: TGF-alpha is present in adult rat brain. Its concentration varies with brain region.

We have recently reported the ability to morphologically fuse the second halves of an invertebrate myelinated giant axon, the median giant axon (MGA) of Lumbricus terrestris (Krause and Bitter, INAS 87: 1471-1475). This morphological fusion is achieved in a few minutes using polyethylene glycol (PEG) in reduced-calcium hypotonic saline. Initial axonal fusion was found at 80-100% in some trials using this technique. We have extended this former work to evaluate both form and function of these fused axons. We now report the ability to regain conduction through the fusion site of formerly severed axons. Using extracellular and intracellular recording techniques, we have found conduction in approximately 20% of fused axons in some trials. Further, we report that fused axons can retain morphological and physiological integrity after 16-26 hours, although physiological integrity is most often present when measured several hours after incubation. Finally, we have extended our study of conditions most conducive to establish high fusion efficiency and are attempting to repair severed axons in vivo using this fusion technology.

This research was made possible by a Texas Advanced Technology Project Grant (#194) and a National Science Foundation Grant (BCS 89-51178) to G.D.B.

EPENDYMAL GLIAL CELL PRODUCTION ALONG THE RETINOTECTAL TRACT PRECEDES OPTIC NERVE REGENERATION IN THE SALAMANDER. L. Gordon-Katz, B. M. Simpson Jr., and B. M. Davis, Dept. of Anatomy & Neurobiology, Univ. of Kentucky, Chandler Medical Center, Lexington, KY 40536;* Dept. of Biological Science, Univ. of Illinois, Chicago, 60680.

Our experiments on regeneration of the optic nerve after spinal cord revealed that large numbers of ependymal axons along the path of the regenerating nerve incorporate 3H-thymidine during the initial phases of axon regeneration. To test the generalities of these results salamander optic nerves were crushed unilaterally followed by 3H-thymidine injections 0-14d or 0-21d post-crush (2-4uCi/g, every other day). At the completion of the injection schedule the entire animal was perfused and autoradiographed. This allowed us to examine the entire course of the regenerative process. In all cases, large numbers of labeled glial cells were seen ipsilaterally along the entire length of the optic nerve. Labeled ependymal cells were seen bilaterally in the diencephalon, particularly in the retinal portions. In some cases, large numbers of labeled ependymal cells were also seen in the contralateral mesencephalon. Labeled neurons were not identified in any of these cases. HRP injections showed that optic axons had not reached the brain by 21 days post-crush. These results suggest that ependymal cell production is induced by damaged axons and confirm the observations of Gaze and Watson (1965) and Stevenson and Yoon (1978). It should be noted that the time course of the presumptive burst of glial/ependymal cell mitosis occurs within the first two weeks post-crush and could be the result of the increased axonal activity following a "conditioning lesion". Supported by NS25617 to BMD.

AXOSOMATIC SYNAPSES INCREASE ON LOCAL SPINAL MOTONEURONS DURING TAIL REGENERATION: DOES SYNAPSE FORMATION PREVENT AXONAL REGENERATION? M. C. Duffy, B. M. Davis, and S. B. Simpson, Jr., Biological Sciences, University of Illinois, Chicago, 60680, Anatomy & Neurobiology, Univ. of Kentucky, Chandler Medical Center, Lexington, KY 40536.

In our previous studies we found that the number of supraspinal axons projecting to the tail increases by 74% during tail regeneration, but only a small fraction of these axons (<4%) enter the regenerated spinal cord (Duffy et al., 1990). We suggest that this may be the result of "synaptic capture" (Bernstein et al., 1978) in which regrowing axons make synapses on denervated targets rostral to the transection, aborting further regeneration. To examine this hypothesis we used morphometric analysis of EM phantograms to test for changes in afferent distribution on motoneuronal rostral to regenerating tail spinal cord. Examination of lumbar IX neurons (presumptive motoneurons) revealed the following properties: 1) Neurons rostral to regenerating tails (n=6) are larger in both circumference and area compared to non-regenerates (n=20) (79.7 pm vs 51.6 pm; 291.9 pm2 vs 124.0 pm2), 2) axosomatic contacts cover a greater percentage of the motoneuron soma following regeneration (32.25% vs 20.8%), and 3) this increased innervation is accomplished by more synaptically boutons rather than larger boutons (21 boutons/cell (avg length = 1.27 pm) vs 9 boutons/cell (avg length = 1.34 pm)). This result suggests that increased axon synaptic activity in the spinal cord immediately rostral to the junction of normal and regenerating spinal cord could be an important mechanism in the inhibition of CNS axonal regeneration.

REGENERATION OF CENTRIFUGAL FIBERS TO THE RETINA IN CICHLID FISH. Anne C. Rusoff. Dept. of Biology, Montana State University, Bozeman, MT 59717.

The optic nerve of cichlid fish contains two populations of axons: the axons of retinal ganglion cells and axons from cells in the diencephalon and telencephalon that project into the retina (centrifugal fibers). The axons of retinal ganglion cells in fish are noted for their ability to regenerate. One factor often implicated in this ability is the continued addition of ganglion cells into adulthood. In contrast all of the retinal fibers projecting cells in the telencephalon and most of those in the diencephalon are born early. This basic difference between the two populations of centrifugal fibers allows for regeneration of the optic nerve allows one to determine if mitotic activity within the cell population is critical for regenerative ability. Previously I have shown that centrifugal fibers did not regenerate (Soc. Neurosci. Abstr. 14; 657 (1988)). Recently I have attempted to confirm this result using two populations of centrifugal fibers that do not regenerate (SOC. NEUROSCI. ABSTR. 14; 657 (1988)). Recently I have attempted to confirm this result using two populations of centrifugal fibers that do not regenerate (Soc. Neurosci. Abstr. 14; 657 (1988)). Recently I have attempted to confirm this result using two populations of centrifugal fibers that do not regenerate (SOC. NEUROSCI. ABSTR. 14; 657 (1988)).
414.7 REGENERATION OF BRAINSTEM-SPINAL AXONS AFTER SPINAL CORD TRANSSECTION IN EMBRYONIC CHICKEN. S.T. Hassan, H.S. Kiesead, and J.D. Stevens, Departments of Anatomy and Zoology, University of British Columbia, Vancouver, B.C. V6T 1Z9.

In the embryonic chicken, brainstem-spinal pathways to the lumbar cord complete their projections around embryonic day (E) 11 of the 21 day developmental period. Recently it has also been shown that brainstem-spinal tracts in the embryonic chicken maintain their capacity for anatomical and functional repair after a complete thoracic spinal cord transection prior to E13. This repair could be attributed either to the developing or to the regeneration of previously axotomized brainstem-spinal pathways. In this study, double labelling was used to distinguish between these two possibilities. On E8-11, the mid-thoracic spinal cord was injected with the first fluorescent tracing dye. One to two days later the mid-thoracic spinal cord was completely transected. After an additional 5 day period, the second fluorescent dye was injected into the cord, caudal to the site of transection. Two days later (on E17-20), the CNS was fixed, frozen sectioned and the brainstem and spinal cord tissue sections were viewed with fluorescence microscopy.

A significant number of double labelled brainstem-spinal neurons were observed following a thoracic cord transection at least as late as E11-12. Each brainstem also contained a small number of cell bodies labelled with either the first or second fluorescent tracer alone (ie. single labelled). The double labelled brainstem-spinal neurons suggest that regeneration of transected projections contributes to the functional repair of spinal cord injuries in embryonic chicken. (Supported by the MRC of Canada and the Rick Hansen Man in Motion Legacy Fund.)

414.8 THE DEVELOPMENT AND REGENERATION OF THE SYMPATHETIC INNERVATION OF THE RAT TAIL ARTERY. C.R. Anderson* and Elisabeth M. McClearn, Department of Physiology and Pharmacology, University of Queensland, Queensland, 4072, Australia.

The sympathetic innervation of the main caudal artery of Wistar rats was examined using the SPG technique for catecholamine fluorescence, and immunohistochemical localisation of tyrosine hydroxylase (TH) and neuropeptide Y (NPY) during normal development and during regeneration following nerve lesions.

At birth, the tail was essentially devoid of sympathetic fibres. By 3d postnatal, small paravascular bundles extended along the proximal caudal artery and the first peripheral fibres were present. By 10d, sympathetic fibres were present over the media of the entire length of the caudal artery but the density of fibres found in the adult caudal artery was not achieved until 45d. At all earlier stages there was a proximo-distal gradient of maturation with a lag of about 7-10d in the most distal regions. Noradrenaline, (as visualised with catecholamine fluorescence), and TH and NPY were all present around the caudal artery from the earliest times that nerve fibres could be detected in the tail.

When the tail of 21d old rats was denervated by freezing all four collector nerves at the level of C7-8 vertebrae, sympathetic fibres around the caudal artery degenerated from 2-3 cm below the level of the lesion. Within 10d following denervation, sympathetic fibres began to reappear around the caudal artery, extending proximo-distally at about 2 mm/day. However, after 50d regenerated fibres were rarely found in the distal 30% of the artery, and this persisted as long as 120d after denervation. In contrast, the density of regenerated terminals in proximal parts of the caudal artery was only slightly lower than that at equivalent levels of control arteries of the same age. The functional properties of the regenerating fibres are currently under study.


To investigate the effect of neonatal peripheral nerve injury on the organization of the spinal cord, we examined spinal reflexes of adult rats (Sprague-Dawley) in which the left sciatic nerve had been cut during the neonatal stage.

After decerebration at the precollicular level under a gaseous anesthesia of O2, N2O and halothane, a laminectomy was performed at the L1-L6 segments. Electrical stimulation was applied to the L5 spinal cord and evoked compound action potentials were recorded in the ventral root of the same segment.

Based on latencies, multiple components of the evoked potentials could be identified: an early monosynaptic reflex and a delayed polysynaptic proximal reflex. In addition, spinobulbospinal reflexes could be identified as judged by the disappearance after spinal cord transection at the C1 level. The sizes of propriospinal and spinobulbospinal reflexes were significantly larger on the lesioned side than on the control side.

These results indicate that neonatal peripheral nerve injury induces alterations in the organization of the spinal cord, suggesting functional plasticity in the spinal cord. (Supported by NIH grants NS21266 and NS1255 and a grant from Bristol-Myers Co.)

414.10 AXONAL REGENERATION AFTER CHRONIC SPINAL CORD INJURY. J.D. Houle, Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205.

The purpose of this study was to determine if neurons retain the ability to regenerate their axonal process for a prolonged period after injury to the spinal cord. True Blue was injected (TB) into one side of the adult rat lumbar spinal cord to label neurons whose axons course through this region. Seven days later these axons were injured by aspiration of the injection site, creating a hemisection cavity. Four weeks later, scar tissue was removed prior to grafting 1 cm long segments of autologous peripheral nerve (PN) to the rostral and caudal surfaces of the lesion cavity. Thirty days later, the distal tip of each of the PN grafts was exposed to Nuclear Yellow (NY) to label neurons that had grown an axon into the graft. Neurons containing both TB and NY were deemed capable of axonal regeneration while a chronically injured state.

There were no TB/NY neurons within the brain, however, numerous dual labeled neurons were identified within Lamine IV through V (excluding IX) ipsilateral and Lamine VI and VII contralateral to the lesion site, with most cells located within 10mm of the lesion. Within individual lumbar dorsal root ganglia nearly 50% of the neurons labeled with NY also contained TB (range 24-74%). This population of regenerating neurons (X=48 range 10-198 per ganglion) had a smaller mean cell area (955 um2) compared to those with TB only (1110 um2). These results indicate that in a chronic spinal cord injury condition certain neurons have the capacity for axonal regeneration long after their initial response to injury. Supported by NIH Grant NS 26380.

414.11 REGENERATION OF HYPOTHALAMIC NEUROSECRETORY NEURONS INTO THE THIRD CEREBRAL VENTRICULAR LUMEN FOLLOWING HYPOPHYSECTOMY--AN ELECTRON MICROSCOPIC IMMUNOGOLD STUDY. WuJen Wu and David E. Scott, Dept. of Anatomy & Neurobiology, Eastern VA Med. School, Norfolk, VA 23501.

The present investigation focusses upon the reorganization and regeneration of neuropeptide containing neurites into the third cerebral ventricle following hypophysectomy. Six male Sprague-Dawley rats were hypophysectomized 4 weeks before harvesting the brains. Hypothalamic tissue was prepared for immunogold staining. Primary antisera against arginine vasopressin (AVP), oxytocin (OXT), and Proopiomelanocortin (TH) were applied with the "on-grid" immunolabelling technique which utilizes secondary antibody-coated colloidal gold probes. Most of the neurites in the ventricular lumen were AVP positive. Gold particles were found within neurosecretory vesicles. Some OXT positive vesicles and AVP positive vesicles were found to be located in the same terminal. Both AVP and OXT positive neurites freely terminate within the third ventricular lumen. No TH positive terminals were observed within the ventricles, although TH neurons in hypothalamus also project to the posterior pituitary. The results of the present study suggest that neurosecretory neurons are able to regenerate and regrow into the ventricular lumen after axotomy. Hence, the CSF may serve as a functional terminal for release of neurohormone. Supported by NIH grant NS 470667.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
415.1

Acetylcholine receptor subunit mRNAs are more abundant in innervated than in denervated muscles and are synaptic compared to extrasympathetic areas of muscle fibers (Merlie et al., J. Cell Biol., 1984; Merlie and Sanes, Nature, 1985). To study the transcriptional basis of these nerve-dependent phenomagens we produced transgenic mice containing an AChR α-subunit promoter fused to the coding sequences of the chicken acetylcholine-esterase (Cae) gene (of β-galactosidase) (lacZ). In vivo transcription and transgenic-CAT fusion, CAt expression was both muscle specific and increased 100X by denervation (Merlie and Kornbauer, Neuron, 1989). To investigate transcriptional differences among and within muscle fibers, we prepared transgenic mice in which the α-promoter fragment was fused to a lacZ gene modified by incorporation of the SV40 large T antigen nuclear transport signal. As determined histochromically, the distribution of LacZ in 3 lines of αLacZ mice was muscle-specific. However, αLacZ mice exhibited pronounced variation among muscles; e.g., tibialis anterior stained far more intensely than soleus. Surprisingly, however, αLacZ expression was not affected by denervation. Furthermore, nuclei within single myotubes were differentially stained; some nuclei were unstained and synaptic nuclei were often more intensely stained than extrapsychatic nuclei. Although measurements of lacZ RNA will be needed to rule out posttranscriptional effects, we suspect that an activity-dependent regulatory element within the α-subunit promoter was suppressed by fusion to lacZ. Thus, muscle-specific and activity-dependent regulation are dissociable. Furthermore, the gene fusion is apparently recognized and expressed differently in different muscles, presumably reflecting unique transcriptional potentials of each muscle. (Support NIH)

415.3

Torpedo acetylcholine receptors (AChRs) stably expressed in mouse fibroblasts L cells form fully functional receptor-channels (Science 238, 1688-94, 1987) and are distributed evenly on the cell surface of the fibroblasts (J. Cell Biol., 94, 1989). AChR surface distribution was determined by labelling briefly with a monoclonal antibody specific to the AChR α-subunit (mAb22) followed by a second antibody conjugated to phycocerythrin with a Zeiss IM3 microscope. A variety of agents were able to induce AChR clusters in fibroblasts. Prolonged incubation of the cells with mAb35, a monoclonal antibody previously shown to prevent antigenic masking, produces small (<2μM) AChR clusters followed by rapid internalization of the clusters. Incubation of the AChR-fibroblasts with Torpedo extracellular conditioned media from an NG108-15 neuroblastoma-glioma cell line, agents which have shown to induce AChR cluster formation in cultured muscle cells, also induce the formation of AChR clusters in these fibroblasts. These clusters are larger than antibody clusters, and range in size from 2-10 μM in diameter. Clusters induced by ECM or NG108-15 conditioned media do not turn over rapidly and the total number of surface AChR clusters does not change. Torpedo AChRs expressed in mouse fibroblasts appear to respond to various clustering agents in a manner similar to that of mammalian AChR in muscle. This result suggests that all these clustering factors induce AChR clustering via direct intermolecular interaction with the AChR molecule.

415.5

Chick muscle-derived agrin (M-agrin) is associated with regions of high acetylcholine receptor (AChR) density in vivo and in vitro. Its antigenic relationship to Torpedo agrin implicates it as a possible synaptic organizing molecule. In vivo synaptic organizing activity resides in the basal lamina. Here we show that M-agrin is detectable by immunofluorescence on unpermeabilized tissue, and is not extractable in 2M EGTA or 0.1% Triton X-100. It is solubilized in 0.2M bicarbonate pH 9, a buffer which also extracts agrin from Torpedo extracellular matrix. The pattern of M-agrin immunostaining in cultures is similar to that of laminin, but it is more restricted. Furthermore, EM-immunocytochemistry demonstrates that M-agrin is a myotube basal lamina component that may secrete agrin-like molecules that become stably associated with the synaptic basal lamina. PHS grant HD 23524 to JRF and NIAH fellowship to EL.

415.2

Agrin, a protein isolated from cholinergic nerve terminals, is secreted by the nerve organ, induces clustering of acetylcholine receptors (AChRs) on cultured chick myotubes. To elucidate the mechanism of agrin's action, we are working to purify and characterize the molecule(s) to which agrin binds as it interacts with myofiber surfaces. We have chosen the C2 mouse muscle cell line as a source of agrin binding molecules. Here we confirm previous findings of Lieth & Hal. SNS Abstr. 15:352 that Torpedo agrin induces AChR clustering on the surfaces of C2 myotubes. Agrin binding sites also become redistributed as part of this response. Clusters of agrin binding sites can be detected histochemically with an immunofluorescent labeling of bound agrin, areocolized with spontaneous as well as agrin-induced AChR aggregates. C2 myotoblasts also express agrin binding sites. NRK fibroblasts, however, do not bind agrin. For C2 myotoblasts as well as myotubes, agrin binding depends upon the presence of extracellular calcium; EGTA abolishes virtually all specific binding much as in chick myotubes (Fallon, this vol.). These results suggest that calcium is important in regulating the action of agrin. Furthermore, the calcium dependence of agrin binding may prove highly useful in subsequent affinity purification strategies.

NIH F32 NS08152 and HD 23924.

415.4
THE POSTSYNAPTIC 43K PROTEIN CLUSTERS MUSCLE NICOTINIC ACETYLCHOLINE RECEPTORS IN XENOPUS OOCYTES. P. B. Scotland, C. W. Luxton, L. Parkes, and H. Reinalter. Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756 and Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

Nicotinic acetylcholine receptors (AChR) are localized at high concentrations in the postsynaptic membrane of the neuromuscular junction. A peripheral membrane protein of M, 43,000 (43K protein) is closely associated with AChR and has been proposed to anchor receptors at postsynaptic sites. We have used the Xenopus oocyte expression system to test the idea that 43K protein clusters AChR. Immunofluorescence of oocytes injected with RNA made from the cDNA of mouse muscle AChR subunits shows that the receptors are uniformly distributed in the surface membrane. Oocytes co-injected with AChR RNA and RNA encoding the mouse muscle 43K protein display AChR clusters of 0.5-1.5 microns in diameter. AChR clustering is not a consequence of increased receptor expression in the surface membrane as determined by measurements of acetylcholine-induced currents. Furthermore, expression of 43K protein does not alter the distribution of lectin binding sites, demonstrating that AChR clustering is not a result of non-specific aggregation of all membrane proteins. The 43K protein is co-localized with AChR in clusters when the two proteins are expressed together and forms clusters of similar size even in the absence of AChR. These results provide direct evidence that the 43K protein causes clustering of AChR and suggests that regulation of 43K protein clustering may be a key step in neuromuscular synaptogenesis.

415.6
NERVE- AND AGRIN-INDUCED ACETYLCHOLINE (ACH) RECEPTOR CLUSTERING IN XENOPUS MULTINUCLEATE MYOTUBES. M. Saito and Y. Kidokoro. Jerry Lewis Center, UCLA School of Medicine, Los Angeles, CA 90024.

Agrin, which was extracted from the basement membrane of the electric organ of Torpedo californica, causes the postsynaptic receptor to aggregate in Xenopus multinucleate myotubes in culture. We tested a hypothesis that agrin released by the motor nerve terminal binds to its receptor in the base- membranen, and subsequently activates the re- ceptor aggregating mechanism in the muscle cell. In Xenopus multinucleate myotubes, both nerve- and agrin induced receptor clustering were, at least partly, due to lateral migration of receptors in the membrane. A blockade of both nerve- and agrin induced clustering presum- ably by immobilizing ACh receptors. Receptor ap- plied in the culture by blocking nerve-induced receptor aggregation and totally inhibited agrin-induced clustering. In contrast, a similar proteoglycan, chondroitin sulphate type A, affected neither of these receptor aggregating processes. Agrin in the culture medium at saturating concentrations partially blocked nerve- induced receptor accumulation.

Our observations are in accord with the hypo- thesis stated above.
415.7

The abstract by Peng and Baker (this volume) discusses the localization of AChRs on normal cultured muscle cells via beads, and its ability to induce the development of postsynaptic specializations. This work shows that in addition to 4D8, mdx and 2F5-1 are also able to induce clustering of acetylcholine receptors (AChRs). Specific receptors for these ligands are known to be present on muscle cells. 4D8, two isoforms of 2F5, and 2F5-1 are unable to induce clustering. Many effects of growth factors are mediated through receptor-associated tyrosine kinase activity. In order to demonstrate the involvement of this activity in AChR clustering, a tyrosine kinase inhibitor, tyrphostin R50864, was applied to the cells prior to bead application. AChR clustering was completely and reversibly blocked by 40 to 80uM R50864. Further work will be carried out to further characterize the specificity of cellular responses to tyrphostins, and to demonstrate tyrosine phosphorylation of growth factor receptors and their substrates in response to this localized application of growth factors. (Supported by NIH grant NS23583 and Muscular Dystrophy Association)

415.8
PARTIAL PURIFICATION OF AN ACETYLCHOLINE RECEPTOR AGGRGATING FACTOR FROM FETAL PIG BRAIN. A. Olick, N. Ye*, E. Dayton and M. McLean. Department of Zoology, University of Maryland, College Park, MD 20742.

It has been shown that developing neurons or neural tissue contain factors that either induce acetylcholine receptor (AChR) aggregation or stimulate the synthesis of AChR on neuronal cultured myoblasts. We have purified a highly purified factor obtained from Torpedo electric organ induces AChR aggregation (Nikitin et al., J. Cell Biol. 103:2471, 1986). ARIA (Udin and Fischbach, J.Cell Biol. 103:168, 1986) and CDPR (New and Mudge, Nature, 323:909, 1986) increase the synthesis of AChR. While relatively crude extracts of mammalian neurons or neural tissues contain AChR aggregating activity, the factor has not been extensively purified.

We report the partial purification of a proteaceous factor from fetal pig brain. This factor induces AChR aggregation in neuronal cultures of rat myotubes within 4 hrs at 30°C. This factor has been purified approximately 500 fold and is associated with a high (100-200 kd) molecular weight protein, active at nanomolar concentrations. The factor elutes from an anion exchange column with a salt concentration between 25 and 125 mM. The most purified fraction migrates on SDS-PAGE gels at 6 major bands from 74 to 170 kd and one band > 200 kd. Activity can be eluted from a small segment of non-denaturing gels containing only a few major protein bands. Protease digestion or incubation at 60°C for 30 min completely eliminates the activity of the aggregating factor.

415.9

During neuromuscular synapse formation in culture the neuron can induce the accumulation or aggregation of acetylcholine receptors (AChR) at the innervation site on myotubes. It has been proposed that the neuron transiently release a factor that locally induces AChR aggregation. In support of this hypothesis, we have shown that local application of a partially purified mammalian AChR aggregating factor induces local AChR aggregation within 4 hrs on growth plated cultured rat myotubes at 30°C.

Primary rat myotube cultures were incubated in 0.3% Diisopropylamine and other non-muscle cells to produce experimentally accessible surfaces. Aggregates formed predominantly on the top surface of these myotubes in response to a 30 min bath applied pulse of a partially purified protein factor derived from fetal pig brain. Local application of the aggregating factor via micropipette to regions of the myotube surface for 30 min or less resulted in AChR aggregation largely restricted to the release site. Similar application of control solutions to the myotube surface had no effect. Further, preliminary studies indicate that this response was not associated with a detectable change in the membrane potential during the period of factor application.

415.10
THE TORPEDO POSTSYNAPTIC 280K PROTEIN IS DYSTROPHIN. S.C. Froehner*, M.I. Butler, A.A. Muma, K. Dougill*, and B. Seaccock*. Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756 and Department of Physiology, University of North Carolina, Chapel Hill, NC 27599.

Purified postsynaptic membranes from Torpedo electric organ contain several peripheral membrane proteins that are thought to play a structural role at the synapse. One of these is a peripheral membrane protein of approximately M, 280,000 (280K protein). Two monoclonal antibodies that recognize different epitopes on this protein were isolated. Immunogold labeling of electric organ with these antibodies shows the 280K protein is restricted to the cytoplasmic side of the innervated membrane in a distribution similar to the nicotinic receptor. By immunofluorescence, both anti-280K mabs stain muscle endplates very intensely and also show specific staining of extrasynaptic membrane. A protein of similar M, in Torpedo postsynaptic membranes is specifically postcytoplasmic. Western blots against antiserum to dystrophin. To examine the relationship between the 280K protein and Torpedo dystrophin, the former was purified by immunofluorescence chromatography. Purified 280K protein was identified on Western blots by both anti-280K mabs and by anti-dystrophin but not by control antibodies. Furthermore, both anti-280K mabs stain normal mouse skeletal muscle, but not skeletal muscle from the mdx dystrophic mouse, which lacks dystrophin because of a mutation in the dystrophin gene. These results indicate that the 280K protein is dystrophin (or a closely related protein) and support the notion that dystrophin may play an important role at the neuromuscular postsynaptic membrane.

415.11
EVIDENCE FOR ASSOCIATION OF THE POSTSYNAPTIC CYTOSKELETAL 58K PROTEIN WITH DYSTROPHIN. M.H. Butler, A.A. Muma, and S.C. Froehner. Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756.

Several peripheral membrane proteins, including the M, 58,000 (58K) and M, 280,000 (280K) proteins are associated with the postsynaptic membrane of Torpedo electric organ and mammalian skeletal muscle. The 58K and 280K proteins are found at high concentrations on the cytoplasmic side of the neuromuscular postsynaptic membrane but are also associated with the extrasympatric sarcolemmal membrane. We recently identified the 280K protein as dystrophin or a closely related protein. Here we report evidence for the interaction of the 58K protein with dystrophin. Because of a mutation in the gene, dystrophin is absent from skeletal muscle of mdx mice. Immunofluorescence staining of mdx skeletal muscle by anti-58K mab is severely reduced when compared to normal muscle. The reduction is selective for the 58K protein since staining of several other cytoskeletal proteins, such as vinculin, and a membrane protein (Na+, K-ATPase) is normal in mdx muscle. Furthermore, preliminary experiments show that immunoprecipitation of Triton-solubilized Torpedo postsynaptic membranes with anti-58K mab also precipitates dystrophin. These results support the association of the 58K protein with the skeletal muscle plasma membrane requires the presence of dystrophin, possibly because the two proteins are present in a complex.

415.12

Competitive interactions between neurons vying for capture of the same target cell are believed to shape patterns of synaptic connections throughout the developing nervous system. Such interactions are poorly understood, in part because it is technically difficult to distinguish the terminals that persist from those that are eliminated. We have addressed this problem by differentially staining the terminals of competing axons using Dil, which fluoresces red (Honig and Hume, J.Cell Biol. 103: 171-187, 1986), and a new lipophilic compound, 4-Di-16-ASP (DIA; Mol. Probes; Eugene, OR, wksh, Abs. S. Soc. Neurosci., 1989), which allows DIA labels cell membranes and can be used as an anterior- or retrograde tracer. In parafformaldehyde fixed tissue, DIA diffuses somewhat faster than Dil; importantly, however, these compounds are segregated even when Dil and DIA labeled axons are interspersed. At embryonic neuromuscular synapses transiently multiply innervated by two different motor axons, one labeled with Dil and the other with DIA, each axon's terminals are similar to growth cones, having many filopodia and only 1-2 small terminal regions per axon. Surprisingly, both axons add discrete synaptic sites at a time, resulting in extensive intermingling of the two inputs. Over time, however, one axon acquires a majority of the synaptic territory, both by expansion of its territory and by the stepwise loss of synaptic sites by the other axon, which is finally resorbed into a retraction bulb process. The junctional complex is presently studying how postsynaptic ACh receptor regions are occupied by competing axons during this period by labeling the same muscles with fluorescent alpha bungarotoxin.
415.13 NEUROMUSCULAR JUNCTIONS ON POLYNEURONALLY INNERTED FIBERS SHOW INCREASED SYNAPTIC DEPRESSION. B. Dunia and A.A. Herrera, Dept. of Biological Science, University of Southern Mississippi, Hattiesburg, MS 39401.

Intracellular recording revealed a growth-associated rearrangement in the innervation of the pectoral muscle of female leech, Hirudo medicinalis. Nerve fibers (90%) were innervated at two different junctional sites. In small frogs (5-16 g), 60% of the fibers were innervated at both sites by the same motoneuron (A/A), while the remaining 40% received inputs from different motoneurons (A/B). In larger frogs (20-47 g), however, 93% of the fibers were A/A. We hypothesize that the development of synapse elimination, competitive interactions in polyneuronally innervated fibers (A/B) result in synapse replacement and conversion to mononeuronal innervation (A/A).

To look for physiological correlates of competition, we recorded curare-blocked end-plate potentials (EPPs) from both junctions on single fibers and calculated the ratio of EPP size (smaller/larger) for each fiber. The mean ratio was 0.60±0.02 (sem) for A/A fibers, and 0.51±0.05 for A/B fibers (p<0.01). When the 50th EPP in a 10 Hz train was measured at the same junctions, the ratio increased in A/A fibers (0.66±0.03) and decreased in A/B fibers (0.48±0.06). The difference was significant (p<0.05), showing that synaptic depression was more pronounced in A/B fibers. These same junctions are being examined for morphological signs of synapse replacement.

415.14 INTERSTITIAL CELLS AT DEVELOPING NEUROMUSCULAR JUNCTIONS. E. A. Connor and R. A. Horowitz*, Univ. of Massachusetts, Amherst, MA 01003.

The structure and function of skeletal muscle depends on its innervation state. In innervated muscle, acetylcholine receptors and N-CAM are found at neuromuscular junctions. In non-innervated muscles, developing or denervated, these molecules are expressed throughout the muscle fiber membrane. The composition of the muscle connective tissue is also dependent on innervation. Interstitial cells accumulate in junctional regions of denervated muscle. In these same regions are found interstitial deposits of segmental fibers, fibronectin, heparan sulfate proteoglycan. We asked whether similar changes in the cellular and molecular composition of connective tissue occur in developing interstitial cells in developing muscles resembles that in denervated muscles; interstitial cells are concentrated in junctional regions. Interstitial cell density was quantitated in embryonic and neonatal muscle of Xenopus laevis at stages X to stage XX. At stage XX, interstitial cells were evenly distributed throughout the muscle. However, at a gradient of interstitial cells in stage XVI muscles; that characteristic features of fibroblasts and resembled the accumulated interstitial cells in denervated muscle in position and morphology. The presence of an interstitial cell gradient in both developing and denervated muscles suggests that the interstitial cells or the matrix molecules that they produce play a significant role in synapse formation.

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS VIII


There are two early-developing groups of dorsal horn interneurons in embryonic rat spinal cord; commissural (C) and association (A) cells that innervate contralateral medial and lateral lateral motoneurons (LMNs), respectively. Dodd et al. (Neuron 1 :105, 1988) have shown that C neurons express the surface glycoprotein TAG-1 early in development. Recently, one of us (E6) has recently found that this molecule is also expressed by A cells. On embryonic day 13 (E13), TAG-1 was expressed intensely on C axons, but some TAG-1-immunoreactive (TAG-1-IR) was also observed on A fibers. By E14, both C and A cells expressed intense TAG-1-IR, with A fibers originating from ventrolateral to the source C fibers in the lateral intermediate. The A fibers were most mutated on the intermediolateral margin of the spinal cord. At E15, TAG-1-IR was more intense on A than C fibers. These cells are in progress to determine if these TAG-1-IR fibers provide pathways for neuronal migration. (Supported by NIH grants NS18585 and NS25784).

416.2 EXPRESSION OF A FASCICLIN-LIKE MOLECULE DURING NEURODEVELOPMENT IN THE COCKROACH. L.S. Yang* and J.L. Denbigh, Dept. of Biology, The Univ. of Iowa, Iowa City, IA 52242.

The presence of molecules involved in specific fasciculation of axons during the development of the nervous system can be identified with monoclonal antibodies. One such mAb, DS-8, was selected for study because it transiently binds to the nervous system of embryos of the cockroach, Periplaneta americana. Like previously described fasciclin the DS-8 antibody was regionally expressed on the surface of and on partial and transverse sections of growing axons in correlation with their pattern of fascistic fasciculation. However, in addition, DS-8 labeled the first axons pioneering a specific subset of CNS axon pathways. In particular, the antigen was present on the first axons of the median fiber tract, the anterior commissure and the posterior commissure. The spatial distribution and temporal expression of the DS-8 antigen suggests that it is playing a role in cell recognition occurring during specific axon fasciculation and in the reading of specific environmental cues during initial axon growth.

416.3 ANALYSIS AND PURIFICATION OF AXON FASCIICLE SPECIFIC ANTIGENS IN THE LEECH. K.K. Ehrenberg and J. Johansen, Department of Zoology, Iowa State Univ., Ames, IA 50011.

Two monoclonal antibodies, lan-3 and lan-4, label a small subpopulation of axons fascicles in the nerve roots and interganglionic connectives of the leech nervous system. The antigens are surface glycoproteins from the leech, H. medicinalis and they recognize three protein bands with molecular weights of 130, 105, and 90 kDa, respectively; whereas lan-4 recognizes a single 130 kDa band (MacKay et al., Science 222 : 789). Purification experiments with the rabbit antibodies have demonstrated that both of these proteins are immunoreactive with antibodies from the leech nervous system. They have been identified as the same proteins by their molecular weight and electrophoretic mobility. The antibodies recognize the antigens in the leech nervous system and are also strongly conserved in the nematode C. elegans.

Immunoprecipitation with lan-3 and lan-4 have demonstrated that the two antibodies label the same 130 kDa protein and thus recognize different epitopes on the same protein. These epitopes are expressed in at least 6 leech species from two different orders. This suggests that the epitopes are conserved in evolutionary and may be involved in neuronal communication. Furthermore, using lectin-affinity chromatography followed by immunoprecipitation, we have purified the antigen homogeneity as judged by SDS-PAGE and silver staining of the gels. We estimate that the antigens constitute roughly 0.004% of total leech CNS protein. These experiments are in progress to purify enough antigen for partial amino acid sequence determination and subsequent cloning of the gene. This was work supported by a Iowa State Biotechnology Grant and a Iowa State University Grant.


New model systems of astroglial fate stress a fixed sequence of steps, a timetable thought to be followed when glial cells are placed into tissue culture. This model predicts that glial support of neurite extension declines with the "age" of the glia, i.e., time in culture. An alternative view is that glial support of neurite growth involves a set of independently regulated events, some of which would be controlled by cell-cell interactions. To test these possibilities, purified mouse cerebellar astrocytes were maintained in vitro for 3 to 6 days to the absence of neurons, explants of pontine nuclei added, and neurite outgrowth quantitated 2 days later. Over time in culture, support of neurite extension declined, from a relatively robust level to negligible levels after 3-4 weeks. Since present studies have shown that neurons induce the differentiation of glial cells (Hatlen J. Cell Biol. 100:394, 1986), we tested the neurons are not required for neurite outgrowth. The explants were harvested on 4 days to 6 weeks, 3 days to 6 weeks. When glial neurons were added at low density (0.1-0.5 x 10⁶/ml), glia and explants showed little neurite outgrowth quantitated 2 days later. Over time in culture, support of neurite extension declined, from a relatively robust level to negligible levels after 3-4 weeks. Since present studies have shown that neurons induce the differentiation of glial cells (Hatlen J. Cell Biol. 100:394, 1986), we tested the neurons are not required for neurite outgrowth. The explants were harvested on 4 days to 6 weeks, 3 days to 6 weeks. When glial neurons were added at low density (0.1-0.5 x 10⁶/ml), glia and explants showed little neurite outgrowth quantitated 2 days later. Over time in culture, support of neurite extension declined, from a relatively robust level to negligible levels after 3-4 weeks. Since present studies have shown that neurons induce the differentiation of glial cells (Hatlen J. Cell Biol. 100:394, 1986), we tested the neurons are not required for neurite outgrowth. The explants were harvested on 4 days to 6 weeks, 3 days to 6 weeks. When glial neurons were added at low density (0.1-0.5 x 10⁶/ml), glia and explants showed little neurite outgrowth quantitated 2 days later. Over time in culture, support of neurite extension declined, from a relatively robust level to negligible levels after 3-4 weeks.
416.5 GLIAL FIBER GEOMETRY ALONE IS NOT SUFFICIENT TO SUPPORT GRANULE NEURON MIGRATION. R.B. Fishman & M.E. Hatten. Ctr. for Neurobiology & Dept. of Pathology, Columbia University, Coll. of Physicians & Surgeons, N.Y., N.Y. 10032.

In vitro heterotypic recombination experiments show that cerebellar granule neurons that migrate across hippocampal astroglia (Gasser & Hatten, PNAS USA 1990, in press). Conservation of glial guidance across brain regions suggests that glia provide a generic, and possibly passive substrate for neuronal migration. In this question, we examined whether geometry of the glial fiber alone is sufficient to support granule neuron migration. Using an in vitro mouse cerebellar system, we tested whether granule neurons could migrate on a) glass fibers of similar geometry to glial fibers, and b) glass fibers of different geometries. Single glass fibers (1-2mm diameters) were obtained by vortexing Whatman glass fiber filters (GF/A). Fibers were coated with either polylysine, membrane from primary astroglia, or membrane from an astroglia tumor cell line, and consequently co-cultured with purified cerebellar granule neurons obtained from postnatal day 4 mice. Although granule neurons bound to glass fibers, and some neurons showed change shapes characteristic of migrating neurons, e.g. elongate profiles and "leading" process extension, neurons did not migrate on glass fibers. Furthermore, preliminary studies show that neurons cultured with lightly fixed (1% paraformaldehyde) glia bound to these glia but did not assume migrating profiles on these. These results suggest that geometry of the glial fiber alone is not sufficient to support migration, but rather migration requires an active interaction between neurons and living glia. Supported by NS15429.

416.6 CENTRAL NEUROITE OUTGROWTH OVER RESTING AND REACTIVE Astrocytes. P. Bovolenta, E. Wando(§) & M. Nieto-Sampedro. Neural Plasticity Lab., Cajal Institute, 28002 Madrid, Spain.

Protoplastic astrocytes are a preferential substrate for neurite extension whereas, after CNS injury, fibrous astrocytes in the glial scar are regarded as a severe hindrance. To examine the cellular basis for this contradictory behavior, we have compared neurite outgrowth from explants of rat retina, septum and spinal cord cultured over purified astroglial substrata (up to 30 days in culture); type 2 astrocytes; the same astrocytes treated with di-butyryl-cAMP or with phorbol ester; astrocytes grown in three-dimensions; membranes from injured spinal cord and injured retina. Neurites from the explants grow over all substrata, excepting myelin. Type 1 and type 2 astrocytes of various ages, flat polygonal astroblasts and astroblasts made to assume a star-shaped morphology with the help of dibutyryl-cAMP, behaved similarly. The walls of a brain injury cavity is, 15 days postlesion, formed to a large extent by reactive astrocyte processes. Membranes prepared from these cells supported neurite outgrowth similarly to cultured astrocytes. In summary, regarding neurite outgrowth, we did not observe significant differences among the various kinds of astrocytes. We conclude that type 1, type 2 and reactive astrocytes are all good substrates. In the case of an open injury, the most likely way in which astrocytes hinder axon regeneration is by "misguiding" the regenerating sprouts away from their targets. (Supported by a grant from the Spanish Science Research Council).

416.7 GROWTH OF RETINAL AXONS ON GOLDEN OPTIC NERVE OLIGODENDROCYTES IN VITRO. M.Bartmeyer, L.Velmeter, G.Giesrich* & C.A.O. Stuermer. Friedrich-Miescher-Lab. der Max-Planck-Gesellschaft, FRG and Universitaet Basel, Switzerland.

Mammalian oligodendrocytes inhibit the growth of neurites (Schwab and Caroni, 1988). Even retinal growth cones of goldfish collapse upon contact with these cells (Bastmeyer et al., 1988). Oligodendrocytes of regenerating fish optic nerves differ from mammalian oligodendrocytes.

1. Gold fish optic nerve axons proliferate in vitro over weeks. After 2-3 weeks in vitro most of these cells express GFAP but also fish myelin glycoproteins detected by the monoclonal antibody (Mab) SDD (Jeserich et al., 1990) and the Mab O4-antigen found on mammalian oligodendrocytes (Sommer and Schachner, 1981).

2. Unlike mammalian oligodendrocytes, fish oligodendrocytes promote the growth of regenerating goldfish optic axons in vitro. Both, regenerating fish retinal axons (Velmeter and Stuermer, 1989) and the fish oligodendrocytes carry the Mab S87-antigen, which has homologies to the mouse cell adhesion molecule L1. In mammals L1 is expressed on Schwann cells, but not on oligodendrocytes. To test whether fish oligodendrocytes are growth permissive for neurites from other species, we cocultured embryonic chick retinal explants and goldfish optic axons. Chick retinal axons grew in high density on the surface of 672 positive goldfish oligodendrocytes. Thus, fish optic nerves through which retinal axons regenerate have oligodendrocytes that: a) at least in vitro promote the growth of axons.

416.8 A METHOD FOR IDENTIFYING MYOTONEURONS FROM INDIVIDUAL POOLS IN CULTURE. V. Roux and D. J. Wiggins, Dep. of Biology and Physiology, Emory University, Atlanta, GA 30322.

We have developed a technique for identifying axonal motoneurons from distinct motor pools in cultured spinal explants. This will enable us to examine in culture the cellular mechanisms underlying the selective reinervation of appropriate target muscles previously observed in vivo.

The lipophilic fluorescent dye 4-Di-10-ASP (Molecular Probes) was found to retrogradely label the cell bodies of motoneurons, as well as their neurites and growth cones which regenerated in culture. Motoneuron and sublaminar muscles of juvenile (6-8cm) axolotls were retrogradely labeled by injection of a 2% solution of 4-Di-10-ASP (95% ethanol) into spinal nerve 17. The nerve was crushed at the injection site to facilitate uptake. The muscles were subsequently transported and could be found within motoneuron cell bodies in 7 days and remained for at least several weeks. Therefore, after 7-20 days the ventral portions of lumbar spinal cord sections containing the muscles were removed and cut into small pieces. These explants were embedded in a collagen-laminin matrix and cultured at room temperature in a modified L15 medium, containing 10% fetal calf serum and other axolotl embryo extract or extract of denervated adult axolotl muscle. Following 4-6 days in culture, labeled motoneurons could be seen within the explants, extending brightly fluorescent processes away from the explant. Since growing processes as well as cell bodies can be labeled, interactions between neurites of identified cells and target tissues in culture can be examined. In addition, since another fluorescent dye, DI, also retrogradely labels neurons and their growing processes in culture, and its fluorescence excitation spectrum is different from that of 4-Di-10-ASP, it should be possible to explore the interactions between neurites from 2 different motor pools and a given target tissue in the same culture.

416.9 INITIAL MOTOR AXON OUTGROWTH. D. Dahmbostel* & K. W. Tosney. Biology Dept., Univ. of Michigan, Ann Arbor, MI 48109.

1) What environmental features determine where motor axons exit from the spinal cord? 2) What interactions exterior to the cord mediate the patterned advance of axons? We are addressing these questions by assessing initial axon outgrowth in thin sections selected from serial, 25µm sections of stage 17 chick embryos prepared as in Tosney and Landmesser (J Histo Cyto 34: 953). 1. Growth cones accumulate at nascent exit points that are spatially predictable but without unique ultrastructural features. Growth cones displace neurophilopitheloid endfeet from the basal lamina, which appears to tear and be carried away by the first growth cones. Cells also exit the spinal cord, but do not preceed growth cones. 2) Upon exiting the spinal cord, growth cones confront populations that provide different environmental for their advance. The posterior sclerotome and sclerotome in the anterior of a segment act as barriers, only the dorsal-anterior sclerotome acts as a pathway (Oakley and Tosney, 1990 NS Abstr.). Axons do have an opportunity to associate with both barrier and pathway tissues: axons exit in both at stage 17 and in each case traverse an adjacent vascularized region. Differences are seen only after contact with sclerote. Axons in dorsal-anterior sclerotome ramify widely, except for a anterior plaque and the very anterior and initial small fascicles. In contrast, axons that enter the more inhibitory environments cluster into larger fascicles and turn within a few microns. These differences in axonal trajectories and in neurite association support a substratum-preference mechanism of guidance, in which entry into a more inhibitory environment discourages advance and promotes closer association among neurites. Supported by NIH grant NS-21308.


We previously demonstrated that peanut agglutinin (PNA) binds to three tissues adjacent to axon pathways in the chick embryo (the posterior sclerotome, girdle precursor, and perinotochordal mesenchyme; PNP) best (Oakley and Tosney) and the more permissive (Oakley and Tosney, NS Abstr. 347, 1988). We report here the distribution of PNA binding as detected with immunohistochemistry following three types of embryonic surroga. Direct PNA binding patterns are retained even in the virtual absence of axons. Following unilateral neural tube deletion, axon pathways to the limb remain PNA-negative, including the dorsal-anterior sclerotome, the plexus region, and the hiatus of the girdle that transitions axons to the limb. We conclude that the differential pattern of PNA binding is independent of motor axon outgrowth. 2) We find that axons turn to avoid the PNP at all stages of outgrowth following neural tube rotations that alter the initial direction of axon outgrowth so as to directly confront motor growth cones with the PNP. We conclude that the dorsal anterior sclerotome is permissive and that the PNP is real latively inhibitory for axon advance. The PNP, like the posterior sclerote and girdle precursor, thus acts as a barrier to axon advance. In contrast, the PNP does not exhibit inhibitory function and does not differentially bind PNA following notochord deletion. The inhibitory function of the PNP correlates with the expression of a PNA binding epitopes. We suggest that general axon pathways may be determined in part by relatively inhibitory characteristics of those tissues that express PNA binding epitopes. Supported by NIH grants NS-21308 and NS-27634.
416.11 DEVELOPMENT OF THALAMOCORTICAL CONNECTIVITY IN VIVO AND IN VITRO. Zoltán Molnár* and Colin Blakemore. University Lab. of Physiology, Parks Road, Oxford OX1 3PT, U.K. We are interested in the mechanisms responsible for the innervation of different cortical areas by particular nuclei of the thalamus. In agagnostically co-cultures, any region of embryonic (E) 16/17 day rat thalamus will innervate any region of cortex (postnatal, P0-8). No positionality effects are observed even when the thalamic explants were given a choice of different cortical targets. McConnell et al. (1989, Science 245:978) showed that subplate neurons pioneer the first axon pathway from the cerebral cortex to the thalamus in cat. We have determined the developmental time course of the establishment of these projections in rat from E13-E17. Tracing with fluorescent dyes from different cortical areas, we observed that these early corticofugal fibers have different projection targets in the diencephalon according to their cortical origin. By E16 the first corticopetal diencephalic fibers have reached their appropriate cortical areas using routes similar to but not identical to those already occupied by the pre-existing corticofugal projections. This coupled scaffold system may be crucial in the development of area-specific thalamocortical connectivity. In culture, E16-E18 cortical sections of thalamic explants but again there is no sign of positional preference. Therefore, we suspect that organized pathways in the extracellular matrix or simple mechanical factors may play important roles in the establishment of thalamocortical connectivity at early stages, when the forebrain is very immature.

416.12 INGROWTH OF THALAMOCORTICAL AXONS INTO EMBRYONIC RAT NEOCORTEX S. Catsalin*and H.P. Killackey. Dept. of Anatomy and Neurosurgery, University of California, Irvine, CA 92717. The growth of thalamocortical axons into rat neocortex was examined using the fixed technique of monolayer explantation of the cortical vesicle. At this stage, a lamina of cells condensing underneath the cortical plate (presumably lamina 6b, Valverde et al., J.Comp.Neural. 290:118, 1989) can begin to separate from the cortical plate by a cell-sparse area. Thalamic axons grow tangentially within lamina 6b as well as halfway to the intermediate zone below, and a dense layer of thalamic axons can be seen growing radially within the cell-sparse zone, between the cortical plate and lamina 6b. By E 20 presumptive layer 6a has begun to differentiate and thalamic axons continue to grow radially through it, but do not penetrate the cell-dense cortical plate. At no point are afferent fibers observed within the marginal zone. Thus thalamic axons are present from cortex from a very early age, and the growing axons are in a position to contact target cells as these laminae differentiate from the cortical plate. (Supported by NSF grant BNS 87-19311).
416.17

COMPLIANCE OF HIPPOCAMPAL NEURONS GROWN ON PATTERNEO MICROCIRCUITS. J. M. Core1, B. C. Wheelis1, and G. J. Brewer2.

1Neuroscience Program, University of Illinois, Urbana, IL 61801 and 2Southern Illinois University School of Medicine, Springfield, IL 62794.

Increasing connectivity of CNS neurons would be facilitated by limiting their number and observing connections formed during directionally restricted growth of their axons and dendrites. Growth direction could be controlled by culturing neurons at low density on patterned substrates. Rat hippocampal neurons were dissociated and grown on these (6000 cells/mm²) in defined medium. Substrate patterning was done by etching poly-lysoe-coated glass substrates with a UV laser through a quartz mask fabricated with electron beam lithography. We have investigated effects of path width and node size on compliance to square patterns.

Path widths were 3, 5, or 10m. Intersecting paths were nodes of 5, 10 or 20μm diameter. Intermodal distances of 80, 120 and 160μm were created. After 3 days of growth, the 60mm pattern lengths showed maximum adhesion (94%) to the 5m path and 20μm node pattern. This was 50% better than the least compliant combination of 3m paths and 5μm nodes.

Somal migration to nodes from paths and from off-path areas was noted from observations over a four day period. These findings demonstrate effective neuron positioning on microcircuits by a process of selective adhesion and migration which will aid in later applications to localize neurons over substrate electrodes. Supported by Pearson Family Foundation and NIH BRSG funds.

417.1


Cultured adrenal chromaffin cells respond to nerve growth factor (NGF) by extending neurites, and after 14-21 days, transdifferentiating into sympathetic neurons. We enzymatically dissociated adrenal medulla and re-suspended the cells in a non-adhesive culture medium consisting of 80% fetal bovine serum, 20% Ham’s F-10, ± 100 ng/ml nerve growth factor. The medium was supplemented with 0.6% glucose, 10% dextan-charcoal-stripped fetal calf serum (Hyclone), 100 μg/ml Gentamycin and 25 mg/ml 75 NGF (Collaborative Research). Using phase optics and time lapse video microscopy, we compared the morphology and motility behavior of growth cones from sympathetic neurons, from naive chromaffin cells and from chromaffin cells that had been exposed to NGF for times up to two weeks. In order to examine growth cones from chromaffin cells at different stages of neuronal differen- tiation, we replated cells after various times in NGF, when these cells are replated they immediately start to extend neurites tipped with growth cones. Results: Growth cones from mature sympathetic neurons do not adhere to the substrate over large areas; they are highly dynamic at the leading edge, with exuberant filopodial and lamellapodial activity. In contrast, after 3-10 days in NGF, chromaffin cells exhibit growth cones that are broad and flat, suggesting a uniformly high adherence to the substrate, and few, if any, filopodia. How- ever, after 14 days of exposure to NGF, many of the replated cells extend growth cones that are indistinguishable from those of mature sympathetic neu- rons. This suggests that a developmental change in growth cone-substratum interaction takes place as these cells differentiate into neurons. (Supported by NSF BNS-8616955 to P.C. and NIH RR00167 to the WRPIC.)

417.2

DIFFERENCES IN CA²⁺ IN HUMAN JOINTS IN GROWTH CONES OF AN IDENTIFIED NEURON: Na⁺-DEPENDENT AMPLIFICATION OF CA²⁺ SIGNALS IN GROWTH CONES. L. B. Jensen, Y. Rehder and S. B. Kast. Dept. of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523.

The kinetics of the responses to perturbations of the intracellular calcium concentration (Ca²⁺) differ greatly in neuronal growth cones (GCs) and in cell bodies. These differences are due to the presence of Na⁺/Ca²⁺ exchangers in cell bodies. The large GCs of cultured Helixoma buccal ganglion neuron 5 were utilized to compare Ca homeostasis in GCs to the soma of the same neuron. The fluorescent Ca²⁺ indicator Fura 2 (K salts) was employed to assay Ca²⁺.

The Ca²⁺ ionophore, 4-bromo A23187, was used to increase Ca²⁺ influx and release from intracellular stores. The continuous influx of Ca²⁺ is responsible for the growth of many GCs because the Ca²⁺ influx triggers an increase in cytosolic calcium without a significant increase in cell volume. In contrast, the Ca²⁺ influx into the soma was limited and the Ca²⁺ signal propagated only over a short distance. These results suggest that the electrostatic fields associated with different tissue culture substrates play an important role in regulating neuronal differentiation in vitro.

417.3

THE EFFECT OF ELECTROSTATIC STIMULATION AND GROWTH CONE CALCIFICATION ON NEURITE OUTGROWTH IN RAT SYMPATHETIC NEURONS. T. K. Guyanites, T. B. Pine, & W.G. Regler1 Caltech, Pasadena CA 91125, AT&T Bell Labs, Murray Hill NJ 07974.

Increasing growth-cone-calcium levels (CaGC) by electrical activity has been proposed as a mechanism to halt neurite outgrowth (Cohan et al., J. Neurosci., 7, 11, p.3588, 1987). We investigated the effect of increased electrical activity and CaGC on the growth rate of cultured neonatal rat superior cervical ganglion neurons (SCGs). These neurons show low levels of spontaneous activity in control saline. We found that stimulation at 10 Hz for 1 hour, in high K+ medium or high K+/high Ca²⁺ medium had little effect upon growth rates. Immediately following the onset of stimulation, CaGC transiently increased to greater than 500nM, but for all conditions tested sustained CaGC remained below 250nM. These results sharply contrast those reported for cultured adult Helixoma neurons where electrical stimulation produced slightly larger CaGC increases but stopped outgrowth. Intriguingly, for SCGs calcium growth rates did not correlate with calcium levels in the range studied (50-250nM). These findings indicate that SCG outgrowth is unlikely to be greatly influenced by electrical activity at frequencies as high as 10Hz. Differences in calcium buffering systems, density or type of voltage-dependent calcium channels, or sensitivity of outgrowth to CaGC between rat SCGs and Helixoma neurons may explain their different responses to electrical stimulation.

417.4


We have used the radiation pressure of a laser trap to manipulate antibody- or protein-conjugated (0.5Σm) latex beads on the surface of embryonic murine cortical growth cones. With the laser trap, beads attached to the cell surface can pull novel membraneous extensions (neopodia) which resemble filopodia in their general form. Release of neopodia from the laser trap resulted in 3 types of response: an instant elastic recoil; or slow but slow; or never. The neopodia formed at the proximal end outward. Thus, mechanical extension of membrane can induce formation of active filopodia, which presumably require actin polymerization. This occurs with greater frequency on active rather than quiescent growth cones, suggesting that specialized structures or conditions exist in the active growth cone.
417.5  

We have investigated the effect of ganglion-derived conditioned medium (CM) on neurite outgrowth in culture using a variation of the protocol reported for *Helixoma* neurons (Vong, et al., J. Neurosci., 1:1008, 1981). Perisomatogaean ganglionic ring sections from six *Aplysia* were placed for 15 min in 1 L-15 media to test the conditioning factor (CF). Culture dishes with poly-L-lysine coated cover slips were exposed to the CM for 24 hrs (1 L-15/dish) of the growth cone medium. Gelman 0.2 j filter and rinsed in 1-15 media. Neurite outgrowth in control and CM dishes was examined after 24 hrs. Only neurites which possessed at least two neurites with a length twice the diameter of the cell body were considered. The total length of the largest neurite of each cell was measured and compared within the two groups. Every cell cultured on the CM showed enhanced neurite outgrowth compared to the control group. For quantification neurite length measurements were made on 15 neurons of each group. Neurite length (Mean ± S.D.) of the control group was 261 ± 89 and 555 ± 107 for the CM group. We concluded that CM released from *Aplysia* ganglia is a growth factor distinct from any found in the hemolymph.

417.6  

The growth cones of living PC12 cells were examined using Video Enhanced Contrast Differential Interference Contrast (VEC DIC) microscopy. During observation, cells were contained in a sealed chamber and could be continuously superfused with medium containing various additives. Thus, this system is useful for observation and pharmacological intervention in vivo of the growth cone.

In the presence of NGF, PC12 cells extended neurites which were tipped by arrow-like growth cones. Depressions visible in the neurite cortex were shown to be cell bodies and the neurite lamellipodium. Upon addition of NGF, growth cones retracted after one minute's exposure to the growth factor. Reattachment included protrusion of many microtubules from the body of the neurite and extension of broad lamellipodium from the leading edge.

The purine analogues 2-aminopurine (AP) and 6-mercaptopurine (6-TG) have been shown to block some but not all of NGF's actions on PC12 cells via differential inhibition of protein kinases (Volente, et al., J. Cell Biol., 109:2935, 1989). Short-term (10 minute) preincubation of PC12 cells with 2-A P blocked NGF-induced growth cone retraction in a dose-dependent manner. The concentrations tested were insufficient to block other PC12 responses to NGF, including neurite regeneration. In contrast, short-term (10 minute) treatment with 6-TG did not inhibit growth cone retraction. Long-term (1 day) treatment of PC12 cells with 6-TG resulted in neurite retraction, compatible with 6-TG's ability to block NGF-dependent neurite outgrowth.

This blockade by one of a family of compounds known to inhibit protein kinases in these cells implies that phosphorylation is involved in the regulation of growth cone motility. We can exclude the NGF-regulated sinapine protein (PKN) from this process since it is relatively specifically inhibited by 6-TG. 2-A, a more general inhibitor, exerts its effects in the growth cone through blockade of kinases distinct from PKN.

417.7  


The time-course of neurite-promoting effects of TPA on sensory ganglia of White Leghorn chick embryos was investigated. Brief exposure of ganglia to 20-200 ng TPA/ml growth media for 10 minutes was sufficient to elicit neurite outgrowth and neurite elongation. The responses were only observed in ganglionic explants that had attached to a collagen substrate prior to TPA treatment. Incubation of neurites with TPA was ineffective. This lack of response to TPA from unattached ganglia was not related to uptake of the phorbol ester. 15 j-phorbol ester lamellar labelling studies revealed that the synthesis of two proteins with molecular weights of 43 and 63 kD was enhanced by TPA treatment. These results suggest that activation of protein-kinase C may be involved in the initial phases of neurite differentiation.

(Supported by NS 21262)

417.9  
CYTOSKELETAL REORGANIZATION IN RESPONSE TO CONDITIONING FACTORS IN REGENERATING HELIXOMA NEURONS. D.L. Kania and C.H. Cohlan. Dept. of Anatomical Sciences, SUNY at Buffalo, Buffalo, NY 14214

Little work has been done to describe the initial events at the cut end of the axon during initiation of regeneration. This study examined the cytoskeletal changes at the end of the axon mediated by conditioning factors which result in the initiation of neuronal growth.

Neurons B19, B5, and B25 were removed from fucal ganglia of the mollusk, *Helixoma*. When plated onto a polylysine substrate in defined medium, a growth cone formed at the end of the axon stump. This growth cone did not advance across the substrate but exhibited lamellipodial and filopodial movements. Fluorescent labeling of actin and tubulin revealed a characteristic pattern of cytoskeletal elements. Thus, these growth cones were indistinguishable from cones formed in the presence of conditioned medium.

The typical response to anatomy is the extension of many new neurites from a single axon stump. After the addition of conditioning factor, many new neurites extended from the periphery of the growth cone formed at the axon stump. The same response was seen for isolated growth cones suggesting a local effect at the growth cone. Cytoskeletal rearrangements of breakdown in the peripheral actin network in the growth cone and accumulation of actin in areas where new neurites formed. Microtubules extended to the edge of the growth cone into an area normally devoid of microtubules.

These data indicate that growth cone formation is independent of the initiation of neurite extension, but is initiated by conditioned medium which signals changes necessary for neurite extension.

(Supported by NIH grant #NS25789)

417.10  
CALCICYCLINE LEVELS DO NOT CHANGE DURING CONTACT MEDIATED GROWTH CONE COLLAPSE. J.K. Ivins, J.A. Raper and R.N. Pittman. Dept. of Pharmacology and Anatomy, L. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The growth cones of chick DRG neurons undergo a collapse of structure when they make contact with the neurites of chick retinal ganglion cells in culture. We have used the calcium indicator dye fura-2 and low light level digital imaging fluorescence microscopy to ask whether this growth cone collapse is mediated by increases in growth cone calcium.

We find that calcium levels in DRG growth cones are very stable during contact and collapse. The possibility remained that small increases in calcium levels below the detection limit of the calcium indicator may be to blame. Detection for our optical system or that this transient of calcium occurs at rates greater than our sampling rates. However, the calcium ionophore ionomycin (1 jM) raised growth cone calcium levels 2-3 fold, but had no effect on growth cone morphology, growth cone motility, or rates of growth cone advance. Furthermore, depolarization of cultures with 1.5 jM KCl caused 2-3 fold increases in calcium levels. These increases were transient in nature, typically lasting 60-90 seconds, but did not affect growth cone morphology, growth cone motility, or rates of growth cone advance.

Growth cone calcium levels also remain stable during exposure to a crude growth cone collapsing activity derived from E10 chick brain membranes. Therefore, changes in growth cone calcium levels do not appear to account for the morphological features of contact-mediated growth cone collapse.

(Supported by NIH grant #22663 and the McKnight Foundation.)
418.1 IDENTIFICATION WITH A MONOCLONAL ANTIBODY OF A TEMPORAL RETINAL AXON PROTEIN IN DEVELOPING CHICK. S.C. McLeon, Univ. of Minnesota, Minneapolis, MN 55455.

In the process of generating monoclonal antibodies to chick retinal axons, one antibody was found that labels by immunohistochemistry most, if not all, axons in temporal retina. The majority of axons in nasal retina were not recognized by this antibody. A similar dichotomy in the staining of neurites from explants of nasal and temporal retina was seen within the first few days in culture. The neurites of living temporal retinal axons were traced for some distance by permeabilizing the membranes suggesting that the antigen is a cell surface molecule. Immunoblots of embryonic retinal tissue revealed a tight band with an approximate mass of 135 kD. The antibody did not bind to the antigen after treatment with trypsin suggesting that it is a protein. The division between the temporal and nasal retina as revealed by this antibody corresponds to the optic fissure. The immunohistochemistry suggested a sharp division between the two sides of the retina in terms of the concentration of this antigen, rather than a continual gradient of the antigen from one side to the other. A sensitive competition based ELISA was developed to quantify the amount of this antigen on axons in different regions of the retina. This confirmed the existence of a step gradient of this antigen. Our working name for this molecule is TRAP for temporal retinal axon protein. TRAP is the first molecule identified in an immunosometime-temporal axis of retinal development. It remains to be determined what role, if any, TRAP plays in development of the pattern of connections between the eye and the brain.

418.3 STRUCTURAL AND FUNCTIONAL ANALYSIS OF LI VIA PROTEOLYTIC CLEAVAGES. A. Aboach and C. F. Legnaguer, Dept. of Neurobiology, Anatomy, and Cell Science, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

In order to determine the location of the LI active site, a series of proteolytic digests were performed on affinity-purified LI. The resulting peptides were analyzed by SDS-PAGE and Western blot using monoclonal antibodies specific for the 135 and 85 kD fragments of the molecule. The cleavage products were then spotted on nitrocellulose-coated dishes, and dissociated mouse cerebellar cells added in order to assess neurite-outgrowth promoting activity (Legnaguer and Leeman, PNAS 84:7753-57, 1987). Elastase digestion of LI resulted in retention of the 135 kD fragment, while eliminating the 85 kD fragment along with the activity of the molecule. Papain digestion eliminated all trace of the 135 kD fragment on Western, but portions of the 85 kD fragment, as well as the activity of the molecule, were retained. These data provide evidence for the importance of the membrane-spanning 85 kD fragment in the function of LI. Supported by NIMH NS25543.

418.4 CELL ADHESION MOLECULES R9D AND NCAM MOVE INDEPENDENTLY IN THE PLANE OF AXON MEMBRANES. J. DRAZBA 1 & V. LEMMON, Laboratory of Neurobiology 3, NINDS, NIH, & Dept. of Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

Neuronal cell adhesion molecules may be functionally cooperative (Kadmon et al., J. Cell Biol. 110:193-208) and may therefore be physically linked. To test this idea, we investigated the localization of R9D (a member of the LI/NL1/NgCAM/G4 family of cell adhesion molecules) and NCAM, two integral membrane proteins, on the surface of chick retinal ganglion cell axons. Polyvalent R9D or NCAM were added to live cultures of B7 chick axons (Drazba & Lemmon, Dev. Biol. 138:82-93) which were subsequently fixed and incubated with monoclonal antibody (Mab) to the other adhesion molecule. The cultures were then treated with separate secondary antibodies in order to visualize the distributions of both cell adhesion molecules. Examination of the axons and growth cones with a Bio-Rad MRC-600 Laser Scanning Confocal Microscope revealed that the R9D or NCAM, exposed to the polyclonal in the living cell, had patched, while the other cell adhesion molecule, labelled post-fixation with the Mab, was distributed evenly over the surface of the same axon. Control cultures fixed before being exposed to both primary antibodies exhibited uniform distributions of both antibodies. These results suggest that R9D and NCAM are free to move independently of one another in the plane of the membrane and so are not necessarily physically linked.

418.6 CELL-TYPE SPECIFIC PERIPHERAL AND INTEGRAL MEMBRANE PROTEINS AND THEIR COMPLEMENTARY MANNOSIDE-BINDING PROTEIN IN THE LEECH CNS. B.N. Cole and D. Zipser, Department of Physiology, Michigan State University, E. Lansing, MI 48824.

In leech neuronal development, sensory afferents entering the synaptic areas of the CNS desfacilitate as they disperse to their connections. This desfacilitation is mediated by the interaction between a mannoside-containing epitope on the sensory-afferent glycoprotein and a putative mannoside-binding protein (Zipser and Cole, Neurosci. Abst. 130, 1989). Sensory afferents express this mannoside-containing epitope on 3 different proteins (109, 103 and 95 kD). We determined the membrane topology of the 3 sensory proteins by phase separation using Triton X-114. The 109 kD protein is found in the aqueous phase, behaving as a peripheral membrane protein, and is expressed early during the time of target recognition (McCaffee et al., Cell, in press). In contrast, the 103 and 95 kD proteins are found in the detergent phase, behaving as integral membrane proteins, and are expressed after axons are extended. The mannoside-binding protein (MBP) distinguishes itself from the putative mannoside-binding protein that interacts with the sensory-afferent protein, we fractionated leech CNS by gel filtration and affinity chromatography. Affinity studies indicate that the sensory protein from CNS homogenized in the presence of mannose, but not galactose, elutes, as expected, in lower molecular weight fractions. Evidence that the 130 kD peripheral sensory protein binds to a mannoside-binding protein on leech membranes.
418.7 DISTRIBUTION OF TENASCIN IN IMMATURE AND MATURE HUMAN BRAIN. R.D. McComb and K.A. Miller. Dep. of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198.

Tenascin (TN) is an extracellular matrix glycoprotein involved in cell adhesion and migration. In this study the distribution of TN was examined immunohistochemically in the human brain (cerebrum and cerebellum) ranging from 17 gestational weeks (GA) to 92 years of age. At 17-26 weeks GA, the intermediate zone of the cerebellum exhibited greater reactivity than the cortical plate. Beyond 30 weeks GA there was diffuse staining of both cortex and white matter which persisted into the first postnatal year. At age 2 a thin layer of the molecular layer (ML) and white matter maintained strong reactivity, but the cortex showed only patchy peripheral staining. In immature cerebellum, intense reactivity was observed in the ML and internal granule layer (IGL). Reactivity in the IGL decreased during the first postnatal year and was not detected at older ages. Reactivity in the ML persisted until the second decade, but was weak or absent at older ages. The dentate nucleus was reactive at all ages, often showing an intense perinuclear pattern suggestive of membrane localization. The temporospatial distribution of TN is compatible with its proposed role in cellular migration in the developing brain. Its persistent expression in adult brain and in reactive astrocytes following brain injury indicates additional functions for this glycoprotein.

418.8 FIBRONECTIN BINDING TO BRAIN SYNAPTOSOMAL MEMBRANE MAY INVOLVE NOVEL MATRIX RECEPTORS. B.A. Bahr, A. Schoors, P.W. Vandenberghe, R. Kebede, G. Keyser, T. Timmers, & G. Van Leuven. CNLIM, University of Ghent, Belgium.

Synapses presumably share properties with other types of cell-cell adhesion contacts. Integrins act as transmembrane links between the extracellular matrix and the cytoskeleton, forming focal contacts with fibronectin (Fn), vitronectin (Vn), laminin, and proteoglycans. 125I-Fn is co-immunoprecipitated with membrane fractions (SPM) with a specific activity of 5-10 pmol/mg protein and this was blocked by RGDS (1 nM), a compound known to bind integrin receptor sites. This result suggested that in addition to the expected interaction between Fn and integrins (pAb) and monoclonal (mAb) antibodies toward human Fn-receptor (FoR) or Vn or VnR recognized by bovine antibodies of 110, 175, 215, and 225 kDa in brain homogenates, but these polypeptides were almost completely absent from SPMs. However, pAb against a fibronectin integrin (Schwartz & Downes) was enriched in a brain extract at the peptide level only at the synapse, in particular on the dendritic spine-like structure identified in synaptosomal preparations, but not on blood vessel lining or on the cells of the cerebral cortex. Thus, an additional interaction betweenFn and integrin receptors was proposed that could reflect the complex extracellular environment that is enriched at the synapse.

Calcium activated proteases (calpains) are concentrated at adhesion sites (Bennett et al., 1984) and it has been established that certain proteins thought to link integrins to the cytoskeleton are substrates for these enzymes. Preliminary studies indicate that calpain I degrades integrin subunits found in brain homogenates and also reduces the concentration of Fn in SPMs. These interactions might provide a route through which increases in intracellular calcium could reorganize neuronal connections.


The monoclonal antibody HNK-1 recognizes a carbohydrate epitope and has been used to label migrating neural crest cells. We report here that HNK-1 also recognizes neuromuscular junctions (NMJ). These observations were made in the double-labeled uncultured muscle masses in whole mount or cryosection with fluorescent alpha-bungarotoxin, nerve terminal dye or Kanaovsky's cholineesterase stain verified HNK-1 binding to NMJ. HNK-1 also stained peripheral nerves, terminals and acetylcholine receptors. The labeling appears similar to that of fluorescent peroxidase agglutinin (FPA), previously shown to recognize the extracellular matrix (ECM) at frog NMJ and myotendinous junctions. Unlike FPA, HNK-1 does not recognize myotendinous junctions but does stain muscle peripherally unmyelinated nerve fibers seen near NMJ and blood vessels. To localize HNK-1 binding sites, we denervated, froze and removed all but one thin horizontal slice containing both NMJ and S-laminin. HNK-1 will stain the synaptic site after nerve and muscle degeneration. Electron microscopy of muscle treated with HNK-1 labeled HNK-1 showed reaction products in the ECM of the NMJ. Results suggest that the carbohydrate epitope recognized by HNK-1 exists in the synaptic ECM. Since this epitope is common to several adhesion molecules, HNK-1 may provide a probe to study the function of carbohydrates in synaptic differentiation and maintenance.


S-laminin, a laminin-like glycoprotein that is concentrated in the basal lamina at the neuromuscular junction (Hunter et al., Nature 338:229, 1989), is synthesized by a site which is observed by electron microscopy to lie outside the synaptic cleft. We used immunolabeling of brain and muscle homogenates (Hunter et al., Cell 59:903, 1989), suggesting that it may be recognized by axons at synapses. However, the distribution of this glycoprotein is not limited to its synaptic location. We have therefore taken two approaches to identify and characterize this protein: (1) we used monoclonal antibodies (mAbs) which were raised against a variety of rat cell lines by immunoblotting with monoclonal antibodies (mAbs) to s-laminin. Second, we constructed an expression vector containing the entire s-laminin cDNA, and isolated stable transfectants in C2 (mouse muscle) and QT6 (quail fibroblast) cells. By immunofluorescence using rat-specific mAbs, both cell lines accumulated s-laminin. C2 cells secrete the recombinant protein and incorporate it into extracellular matrix after they fuse to form myotubes. When immunoprecipitated s-laminin with subunit-specific antibodies to analyze the oligomeric structure into which it is assembled. Laminin is composed of homologous A, B1, and B2 subunits; s-laminin is most closely related to the B1 subunit. Antibodies to s-laminin corecipitate laminin B2 and an A-like subunit, whereas antibodies to B2 precipitate B1, B2, s-laminin, and an A-like chain. These results support immunostaining data (Sanes et al., submitted) suggesting that s-laminin and B1 can substitute for each other in a trimer with A and B2. We are now purifying s-laminin to test this model and to study interactions of neurons with the complex. (Supported by NIH and Monsanto.)

418.11 LAKININ AND S-LAMININ ARE PRODUCED AND RELEASED BY ASTROCYTES. SCHWANN CELLS AND SCHWANNOMAS. A.Chiu, A. Espinoza, R.Cole, S. Loera & J. de Vallis. Beckman Res. Inst. of City of Hope, Duarte, CA & UCLA, Dept. Anat. & Cell Biol. L.A., CA. Laminin, a potent promoter of neurite outgrowth, is present in all extracellular matrices (ECMs). In contrast, s-laminin, a homolog of laminin, is highly localized at the neuromuscular junction. While the expression of ECM proteins in serum-free astrocyte cultures is not known, it is clear that these two ECM components have been well documented in situ. The sources of these molecules is unclear. We report that astrocytomas produce and release laminin and s-laminin, but only incorporate the former into an ECM. Immunohistochemistry (IHC) shows that s-laminin is present in cultures of Schwann cells or Schwannomas, and laminin and s-laminin immunoreactivity is present within cells and in the conditioned medium. These results indicate that certain cellular components of cells secret and cells lines can produce and release both synaptic and extracellular components of the ECM. Assembly of these different molecules into an organized ECM may require additional factors or interaction with neurons. Supported by March of Dimes 5-608 and NSF #BSR-8617043 (AUC), and NICHD MH06576 (Jdev).

418.12 LOCALIZATION OF A PLASMINOGEN ACTIVATOR AND ITS INHIBITOR AT ADHESION SITES OF CULTURED RAT MYOTUBES. G.M. Demytenko and M.B. Clark. Deps. of Neurology and Anatomy, Univ. of Maryland Sch. of Medicine, Baltimore, 21201.

Cultured neonatal rat myotubes cluster AChRs, develop cytoskelental and membrane specializations, and organize extracellular matrix molecules which they adhere to the substrate. Since the protease plasminogen is known to be involved in extracellular matrix croslinking, we have investigated whether controlling factors, such as plasminogen activators (PAs) or their inhibitors (PAs) were positioned to influence the modification of the extracellular matrix at sites of cell-substrate adhesion. We used sequential enzymatic digestion and immunofluorescence microscopy to identify a urokinase - like PA (uPA) as well as a plasminogen activator inhibitor (PAI-1) immunoreactive at AChR-containing sites. Adhesion sites isolated with saponin demonstrated uPA immunoreactivity in a linear pattern coincident with AChR-rich domains. PAI-1 immunoreactivity was present in the adhesion substrate but apparently absent from the adhesion sites. When the isolated myotube - substrate adhesion sites were further treated with Triton X-100 to remove extracellular matrix, dephosphorylation was seen at the adhesion sites, while PAI-1 was seen in the surrounding culture substrate and appeared reduced at the adhesion sites. Thus, PAI-1 is generally distributed in the extracellular matrix which is dephosphorylated by plasminogen activator, while PAI-1 is focally positioned to affect changes in the extracellular composition of myotube - substrate adhesion sites. Supported by NS01255 to GMD and by PVA Grant 645 to MBC.
418.13

CNLM, University of California, Irvine CA 92717 USA.

The calcium-dependent protease calpain is thought to play a role in the elaboration of structural plasticity in both erythrocytes (Siman et al., 1987) and neurons (Lynch and Baudry, 1984). We have investigated aspects of its biochemistry in crude synaptic plasma membranes prepared from rat telencephalic tissue. Western blot analysis, utilizing a polyclonal antibody generated against purified calpain I isolated from rat erythrocyte cytosol, revealed that the expression of an apparently activated form of calpain is developmentally regulated at the neuronal membrane during a period of considerable structural plasticity. Similar analysis demonstrated that a principle substrate, namely spectrin, was available at the submembranical surface and susceptible to the proteolytic action of calpain throughout the postnatal developmental period studied. The action of calpain was found to cause a loss in the expression of a retarded number of membrane associated components. Nearly all of these were relatively large molecular weight Concanavalin A-binding glycoprotein and included the cell adhesion molecule N-CAM. The present findings are consistent with and support the postulated role of calpain in facilitating membrane plasticity.

418.15

MICROFIBRILLAR ADHESION COMPLEXES OF DEVELOPING NEURITES: LINKAGE TO THE CYTOSKELETON. S.A. Evans & V.J. Klein.
Northwestern Univ. Inst. for Neuroscience, Evanston, IL 60208

The cytoarchitectures of developing nerve cell surfaces contains microfibrillar specializations that constitute adhesion complexes (PAS 52:5206). These somewhat irregular microfibril (8-13 nm wide, varying lengths) can form trans-junctional adhesion complexes (TACs) at nascent junctions as well as connect the cell surface to the substratum. For avian retina cells (Dev.Brain Res. 51:209), TACs and other surface microfibrils have been found to be multimeric and include components of adhesion such as heparan sulfate proteoglycan, as e.g. the current study extends observations of cell surface adhesive microfibril to cultures of rat fetal brain cells and several cell lines including human. Of particular interest, TACs and other adhesive microfibrils have been found resistant to mild detergent treatments that extract membranes and cytosol. Whole mount EM of extracted cells showed that the microfibril were linked to the cytoskeleton, directly supporting the hypothesis that adhesive molecules establish trans-membrane linkage with intracellular elements. Such complexes conceivably could have signal transduction capability. Molecular analysis showed that, after extraction, the microfibril still were labeled by antibodies to adhesion. Studies also were done showing that some adhesive microfibrils include amyloid precursor protein (APP), a molecule closely associated with the pathogenesis of Alzheimers disease.

418.16

MONOCLONAL ANTIBODY KHR1-5 TO HAIR CELL STEREOCILIA IMMUNOSTAINS A CYTOSKELETAL STRUCTURE IN CULTURED FIBROBLASTS. M.Pok, J.W.Horne*, T.S.Nair*, T.E. Greer*, R.A. Altshuler, Kresge Hearing Research Institute. Univ. of Michigan, Ann Arbor, MI 48109

A murine monoclonal antibody developed after immunization with chick basilar papilla, guinea pig outer hair cells and frog lateral line specifically labels stereocilia in the inner ear. In the present study this antibody was applied to cultured fibroblasts where it labeled a fibrous network in the cytoplasm. Double labeling with phallloidin (marking filamentous actin) showed that KHR1-5 labeling was distinct from but associated with f-actin. Immunoelectron microscopy was then used to study ultrastructural localization. Permeabilized fibroblasts were incubated with KHR1-5 antibody and antigenic sites were visualized by 5 nm immunogold particles. Specific labeling was distributed in clusters in close proximity to 200-300 nm fibers. Since both hair cell stereocilia and fibroblast protactin are recognized by KHR1-5, they may have this cytoskeletal structure in common. Its characterization should be facilitated by the use of cultured cells. (Supported by NIH grant NS 07585 and Deutsche Forschungsgemeinschaft - Postdoctoral Fellowship for M.P.)

418.17


CD4 has recently attracted much interest since it is the receptor for Acquired Immunodeficiency Syndrome (AIDS) virus (HIV). It is known that HIV can cross the placenta and infect the fetus. In order to investigate the possibility of a direct infection of fetal brain, we performed immuno-histochemical localization of CD4 both at light and electron microscope in cultures of fetal brain from 8 to 20 weeks of gestation. Results showed that CD4 was present in both cytoplasms and cell membrane of neurons in the first 4 weeks in vitro. Later, CD4 was localized also in the cytoplasms of some, not all astrocytes. Immunoprecipitation studies showed the presence of a protein of molecular weight slightly lower than normal (45 Kd versus 60 Kd). These results were confirmed by molecular biology studies, which showed the presence of a transcript corresponding to a truncated form of the protein (lacking of the gp120-binding site) in early stages (from 12 to 16 weeks of gestation), whereas the complete protein was found in older fetal brains.
**THURSDAY AM**

**PRESYNAPTIC MECHANISMS IV**

1013

**419.1**


Presynaptic facilitation of the synapses between the siphon sensory and the Gill motor neurons evoked by 5-HT involves at least two mechanisms: a first process that is 5-HT dependent and contributes to the presynaptic action potential and a second process that may involve direct modulation of the transmitter release machinery (Gingrich and Byrne, 1985; Hochner et al., 1986). 5-HT can increase the rate of spontaneous neurotransmitter release at cultured Aplysia sensory-motor synapses by a mechanism that seems to be independent of changes in the presynaptic levels of Ca²⁺. This modulation of the secretory mechanism by presynaptic release may reflect activation of the second process by 5-HT (Dale and Kandel, 1990). While CAMP can inflate the terminal, further action in the presynaptic terminal may also be involved: possibly protein kinase C (PKC). We show here that in Aplysia saline, containing zero Ca²⁺, activation of PKC by application of phorbol dibutyrate (PDBu, 150 mM) to Aplysia sensory-motor synapses in culture increases the occurrence of mEPSPs in a dose dependent manner (100 nM PDBu caused fold increase in the frequency of mEPSPs) without altering the amplitude of the mEPSPs. In the inactive isoform, oPDBu, had no effect. Based application of the generalized kinase inhibitor H-7 (200 μM) partially reversed the enhancement of spontaneous release by both 5-HT and PDBu. This suggests that activation of PKC may contribute to the Ca²⁺ independent enhancement of spontaneous release that accompanies the effects of 5-HT. Therefore, PKC may contribute along with CAMP to the secretion or loss of neurotransmitter or to the secretory process of spontaneous facilitation.

**419.2**


An Aplysia 17 motor neuron co-cultured with a single presynaptic sensory-motor cell exhibits spontaneous miniature EPSCs which can be used to assay the release process during the induction of the activity-dependent increases in transmitter release (Kandel and Ezrin, 1990). Cultured sensory-motor synapses undergo homosynaptic depression with low frequency stimulation (<1 Hz), PTX with high frequency stimulation (20 Hz) for 20 s, and longer lasting depression when the neuron is given temporarily paired with the 5-HT. We observe that neither the frequency of spontaneous release during these various activity-dependent forms of synaptic depression (not as might be predicted if they involve depletion or mobilization of synaptic vesicles (Gingrich and Byrne, 1985; Hochner et al., 1986). We show that activation of a presynaptic component of secretion that is characteristic of the spontaneous release process.

**419.3**

PRESYNAPTIC INHIBITION INDUCED BY G PROTEIN ACTIVATION AT THE SQUID GIANT SYNAPSE. S.D. Hess and J.D. Augustine. Univ. of Southern California, Los Angeles, CA 90089 and Max Planck Institut, Goettingen, FRG.

Guinea fowl catecholaminergic fibers were microinjected directly into the preterminal dendrites of squid (Loligo pealei) and L. opalescens to examine the possible role of G proteins in transmitter release at the GABA-gamma-S irrevocably reduced post-synaptic currents (PCs) evoked by presynaptic action potentials by 70 to 85% (n=18). The dose-response for this effect is shown in Figure 1. GABA-gamma-S depolarized the presynaptic terminal 9.5 mV and decreased the action potential amplitude, while GABA-gamma-S at 70 mV GABA-gamma-S reduced transmitter release by only 34.9% (n=2). These results suggest that activation of one or more presynaptic G proteins by GABA-gamma-S inhibits transmitter release. One component of this inhibition is caused by a decrease in the presynaptic membrane and action potentials.

Supported by NIH NS21624, NS08392 and MPI funds.

**419.4**

HISTAMINE STIMULATES SYNAPSES I. PHOSPHORYLATION AND CATECHOLAMINE RELEASE IN BOVINE ADRENAL MEDULLARY CHROMAFFIN CELLS. J.A. Firestone, M.D. Browning. Dept. of Pharmacology, Univ. of Colorado Health Sci. Center, Denver, CO 80262.

Adrenal medullary chromaffin cells have classically been used as a model for the study of neurotransmitter secretion. Stimulation of histamine receptors stimulates catecholamine (CA) release from primary cultures of bovine chromaffin cells. Histamine also stimulates CA release from these cells in a time and dose-dependent manner. We have shown (Haywood et al., J. Neurosci. 13, 1993) that nicotine stimulates incorporation of [3H]-norepinephrine into [3H]-noradrenaline-labeled cells. Histamine significantly potentiated nicotine-stimulated release of CA. We hypothesized that histamine would also increase in synaptic vesicle II (PHIP) phosphorylation of synapsin II and increase in the release of nicotine. We report here that histamine stimulates [3H]-incorporation into synapsin II. The time course of histamine-stimulated phosphorylation of synapsin II closely parallels the histamine-stimulated release of CA. The magnitude of histamine-stimulated secretion is less than that stimulated by nicotine. Interestingly, the extent of histamine-stimulated phosphorylation of synapsin II is also less than for nicotine. After two minutes of treatment, histamine-stimulated phosphorylation of synapsin II is 40% of that produced by nicotine, while release stimulated by histamine is 30% of the release stimulated by nicotine. Thus, there is a strong correlation between histamine-phosphorylation and CA release in response to two distinct secretagogues. One-dimensional phosphopeptide maps of synapsin II yielded a characteristic 18kD phosphopeptide. This portion of the molecule is known to contain the site phosphorylated in vitro by the cAMP-dependent protein kinase and by Ca²⁺/calmodulin kinase I. The determination of the kinase that is active in the present in situ analyses is the subject of current investigation. The data presented here suggest that histamine II (PHIP) may play a role in CA release from bovine adrenal medullary chromaffin cells.

**419.5**

IMAGING CALCIUM TRANSIENTS IN PATCH CLAMPED CHROMAFFIN CELLS. J.J. Augustine and E. Neher. Max Planck Inst. for Biophysical Chemistry, Goettingen, FRG.

We have used video microscopy to examine the intracellular Ca concentration ([Ca]ᵢ) changes produced in single, cultured chromaffin cells by depolarization. Brief (20-50 s) depolarizing pulses applied via a patch pipette, caused measurable changes in the fluorescence of fura-2 (0.1-0.5 μM) when the depolarization occurred in the voltage range of the Ca channels (Ca <20 to +50 mV). These signals were due to changes in [Ca]ᵢ because they were abolished by inclusion of 10 mM GTP in the internal dialysis solution. The changes in [Ca]ᵢ that increased strongly to 0.5 μM in the region just beneath the plasma membrane and smallest in the central cytoplasm of the cell. Closure of the Ca channels, either by increasing the [GTP] or the depolarization of the membrane potential, caused the spatial gradients to collapse very quickly (half-life < 200 ms or less). These results indicate that Ca channels closed after a further depolarization of the membrane potential, as the primary source of the [Ca]ᵢ transient produced by depolarization and that Ca (and/or Ca-loaded fura-2) rapidly diffuses into the cell. Transient [Ca]ᵢ gradients may play an important role in these cells during depolarization-induced secretion.

**419.6**


We have used video microscopy to examine the intracellular Ca concentration ([Ca]ᵢ) changes produced in single, cultured chromaffin cells by depolarization. Brief (20-50 s) depolarizing pulses applied via a patch pipette, caused measurable changes in the fluorescence of fura-2 (0.1-0.5 μM) when the depolarization occurred in the voltage range of the Ca channels ([Ca]ᵢ <20 to +50 mV). These signals were due to changes in [Ca]ᵢ because they were abolished by inclusion of 10 mM GTP in the internal dialysis solution. The changes in [Ca]ᵢ that increased strongly to 0.5 μM in the region just beneath the plasma membrane and smallest in the central cytoplasm of the cell. Closure of the Ca channels, either by increasing the [GTP] or the depolarization of the membrane potential, caused the spatial gradients to collapse very quickly (half-time < 200 ms or less). These results indicate that Ca channels closed after a further depolarization of the membrane potential, as the primary source of the [Ca]ᵢ transient produced by depolarization and that Ca (and/or Ca-loaded fura-2) rapidly diffuses into the cell. Transient [Ca]ᵢ gradients may play an important role in these cells during depolarization-induced secretion.

**419.7**


An Aplysia 17 motor neuron co-cultured with a single presynaptic sensory-motor cell exhibits spontaneous miniature EPSCs which can be used to assay the release process during the induction of the activity-dependent increases in transmitter release (Kandel and Ezrin, 1990). Cultured sensory-motor synapses undergo homosynaptic depression with low frequency stimulation (<1 Hz), PTX with high frequency stimulation (20 Hz) for 20 s, and longer lasting depression when the neuron is given temporarily paired with the 5-HT. We observe that neither the frequency of spontaneous release during these various activity-dependent forms of synaptic depression (not as might be predicted if they involve depletion or mobilization of synaptic vesicles (Gingrich and Byrne, 1985; Hochner et al., 1986). We show that activation of a presynaptic component of secretion that is characteristic of the spontaneous release process.
419.7
DEPOLARIZATION INDUCED CHANGES IN INTRACELLULAR FREE CALCIUM IN INDIVIDUAL NERVE ENDINGS FROM THE NEUROHYPOPHYSIS. E. Steunkel, Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109.

Although neurotransmitter or neurohormone release is dependent on depolarization-induced changes in intracellular free Ca(2+)-anatomical limitations of the synapses to nerve terminals have been examined directly and the characteristics of such changes at single axon endings. Using a-amino-terminal isolated from the neurohypophysis of the rat, changes in Ca(2+) in individual nerve endings were examined in response to depolarizing stimuli. (Ca(2+)) was monitored by dual wavelength microfluorimetry of cytoplasmic fura-2. Injection of membrane depolarization by elevated concentrations of K+, led to a rapid, dose-dependent (EC(50)=150 mM increase in [Ca(2+)](150) M increase at EC(50)) that was sensitive to block by the dihydropyridine (DHPl nicardipine (EC(50)=2.1 mM, DBB) and by the ionotropic Ca(2+) channel blockers Ca(2+) and La(2+) and the non-selective GVA was without effect on Ca(2+). Depolarization of the nerve endings by yohimbine resulted in a similar rapid, DHPl and DHBB-sensitive increase in Ca(2+). Close correlation was found between the K+-induced increase in Ca(2+) and the reported values of vasopressin secretion obtained on populations of isolated terminals. K+-induced increases in Ca(2+) were maintained throughout a sustained depolarizing stimulus and were dependent on sustained influx of Ca(2+) via DHBB-sensitive Ca(2+) channels. Recovery to basal Ca(2+) followed removal of the depolarizing stimulus with the rate of recovery being dependent on the duration of the depolarizing stimulus. Recovery of Ca(2+) was partially blocked by metabolic inhibitors and La(2+) but was unaffected by removal of extracellular Na(+) or application of vanadate thereby suggesting a dominant role for Ca(2+)/ATPase activity over Na(+) /Ca(2+) exchange in recovery.

419.8
A COMPARISON OF THE SUBSEQUENT KINETS OF Η-Glutamatergic AND ENDogenous Glutamate RELEASER FROM NeURONS. T.J. Tuner and K. Dunlap, Dept of Physiology, Tufts University School of Medicine, Boston, MA 02111.

The aim of this study is to characterize the release of "fast" transmitters, such as glutamate, and "slow" transmitters, like Substance P (SP), from intact neurons on a time scale approaching that on which synaptic transmission occurs. To accomplish this, we are using a superfusion device with 50 μL superfusate volume to stimulate release from sensory neurons cultured from embryonic chick dorsal root ganglia; such neurons are capable of releasing both gluta and SP. The neurons were perfused with a saline containing 5 mM glucose and 4 μg/ml of the μ-opioid receptor agonist (D-fenmetazole) to suppress release. A synaptosome fraction was obtained from the superfusate obtained from superfused dorsal root ganglia were collected, and the HPLC-EC was performed. The results show that, as expected, Substance P release is inhibited by veratridine while glutamate release is increased by veratridine. These results indicate that the release of glutamate is time-dependent and that the release of Substance P is not time-dependent.

419.9

There is currently very little information about presynaptic GABA receptors modulate GABA release in the CNS. It has been demonstrated that the GABA agonist muscimol was able to inhibit GABA release from synapses obtained from rat cortices, whereas the GABA antagonist baclofen was able to inhibit GABA release from synapses obtained from the substantia nigra pars reticulata (Pittaluga, E. J. Pharm., 140, 149, 1969). Aims to demonstrate autoreceptor regulation of GABA release in the VP. Rats were chronically implanted with a guide cannula 3 mm above the VP. Post-surgery (7-8 days) each rat was placed in a dialysis box and a dialysis probe was inserted into the VP via the guide cannula. After a 3 hr equilibration period, 5×20 min baseline samples were collected and then dialysis buffers changed to one of the following stepwise increases in agonist concentration: a) dialysis buffer, b) dialysis buffer + 0.1 μM, 1 μM, and 10 μM muscimol, or c) dialysis buffer + 1.0 μM, 10.0 μM, and 100.0 μM baclofen. Each concentration of agonist was perfused for 80 min; samples were collected every 20 min. Artificial CSF, at a flow rate of 2.13 μl/min was used for dialysis. Using microdialysis, which has been shown to be a non-invasive and non-intrusive method of measuring neurotransmitter release, we can measure the release of GABA and other neurotransmitters from the VP using a microdialysis probe. In this study, we used microdialysis to measure the release of GABA from the VP in freely moving rats. GABA release was measured in the following concentration ranges: 0-10 μM, 10-100 μM, and 100-1000 μM. The results showed that GABA release was inhibited by muscimol and that the inhibition was dose-dependent. However, baclofen did not inhibit GABA release.

419.10

Subcellular mechanisms involved in the modulation of acetylcholine (ACh) release in the central nervous system are still not clearly understood. The object of these studies was to examine the role of presynaptic K+ channels in the modulatory process of ACh release in the hippocampus. Using whole-cell voltage clamp, veratridine was applied to slices to increase K+ currents. The results showed that veratridine increased ACh release from hippocampal slices, and that the increase was not due to a direct action on NMDA receptors. Furthermore, the results showed that veratridine increased ACh release from hippocampal slices, and that the increase was not due to a direct action on NMDA receptors. Moreover, the results showed that veratridine increased ACh release from hippocampal slices, and that the increase was not due to a direct action on NMDA receptors.

419.11
EVIDENCE THAT TETRAETHYLMERONIUM SENSITIVE K+ CHANNELS CONTRIBUTE TO PRESYNAPTIC SPIKE REPOLARIZATION AND CONTROL OF TRANSMITTER RELEASE IN HIPPOCAMPAL SLICES. C. Paulson, M. Restat and J.J. Granit, Inst. of Neurology, Karol Johns, g. 47, 0162 Osl, Norway.

Presynaptic K+ channels can indirectly control transmitter release by determining spike duration and hence Ca influx. In the hippocampus, the K+ channel blocker tetraethylmeronium ion broadens the presynaptic fiber volley and increases synaptic potentials (Hass et al., 1983:36(11)). It has also been suggested that a K+ channel-sensitive activated current could contribute to presynaptic spike repolarization, thus causing a feedback control of Ca influx and transmitter release (Storm, Brain Res. 1987:435:387). To test this and related ideas, we have started to measure the effects of the K+ channel blocker tetraethylmeronium ion on the excitatory postsynaptic potential (EPSP) in the CA1 field of rat hippocampal slices. In submerged slices (23-30 °C) stimulation of CA1 str. radiatum fibers produced field potentials of the EPSP layer. The response consisted of a fiber volley (amplitude 0.2-1.2 μm, duration 1.2-1.8 ms) followed by a field EPSP (0.3-1 mV). The EPSP could be blocked by kynurenic acid or the Ca channel blocker verapamil. The results show that tetraethylmeronium broadens the fiber volley of presynaptic origin (n=100), whereas Ca-free medium with 2 mM Mn revealed no effect of TEA. These results suggest that TEA-sensitive K+ channels in presynaptic spike repolarization and the control of transmitter release.

[Supported by the Norwegian Medical Research Council (RMF/NAV)]
419.13
PHARMA COLOGICAL AND FUNCTIONAL CHARACTERIZATION OF AN ALPHA ADRENOCEPTOR IN AREA CA1 OF THE RAT HIPPO CAMPUS. D.V. Mulyina and V.A. Droz. Depar tment of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, California 94305.

Previous work has shown that norepinephrine (NE) decreases synaptic inhibition in the rat hippocampus via activation of an alpha adrenoceptor. To further characterize the subtype of alpha adrenoceptor involved in mediating this action, we examined the actions of a large number of known alpha adrenoceptor agonists on the size of the evoked inhibitory postsynaptic potential. We found that this is at alpha adrenoceptor of unusual pharmacology. This particular adrenoceptor is activated by phenylethanolamines with a rank order as follows: 6-flupropiphrpine OR epinephrine > fenfluramine = NE > phenylephrine = α-tocopherol. The selective alpha-2 agonist 9-methyl NE also possessed significant activity at this adrenoceptor. Initial experiments with several agonists selective for alpha-1 (prazosin, yohimbine) adrenoceptors suggest that the predominant subtype of adrenoceptor involved is an alpha-2. Interestingly, compounds of the guanidinium (e.g., guanabenz, guanadrel) or imidazoline class (e.g., clonidine, oxymetazoline, clonidine, para- amino-clonidine) were either ineffective or produced a weak excitatory (alpha agonist) response against this adrenoceptor. These results are in agreement with several recently published studies indicating that the imidazolines and phenylethanamines interact differently with alpha adrenoceptors, and that many actions of the imidazolines previously ascribed to alpha adrenoceptors may be attributable to a newly discovered imidazoline/guanidinium receptor site distinct from alpha adrenoceptors. In summary, our results confirm that an alpha adrenoceptor mediates the long-lasting action of NE in the rat hippocampus and suggest that this adrenoceptor is of the alpha-2 subtype. We have found, however, that only catechol-type compounds of the phenylethanolamine class behave as full agonists at this particular alpha adrenoceptor.

D.V.M. is a Lucile P. Markey Scholar and this work was supported in part by a grant from the Lucile P. Markey Charitable Trust. V.A.D. is supported by the NIMH. The authors acknowledge the gift of 6-flupropiphrpine from R. K. Kirk (NIDDK).

419.15

Glutamate is presumed to be a precursor for glutamine used as transmitter. Glutaminase catalyzes the cleavage of glutamine to glutamate. Glutamate inhibits glutaminase. To investigate the role of glutamine as precursor for glutamate this study examines the effects of Glutamate on glutamatergic excitatory synaptic transmission.

Hole cell patch voltage clamp recordings were obtained from large cerebellar neurons (Purkinje cells) in primary dissociated tissue culture. Stimulation of nearby neurons with an extracellular electrode produced monosynaptic excitatory currents identified by their ability to follow stimulation at 20 Hz. Glutamate (10 mM) reversibly abolished evoked excitatory postsynaptic currents (EPSCs) but did not abolish spontaneous miniature EPSCs. TTX (5 μM) and Mg2+ (10 mM) also abolished evoked EPSCs and did not affect spontaneous miniature EPSCs, indicating that the latter are due to spontaneous release of transmitter from presynaptic terminals.

The lack of an effect of Glutamate on spontaneous transmitter release indicates that abolition of evoked EPSCs by Glutamate is not due to interference with glutamate as a precursor for glutamate. Possibly, Glutamate abolishes evoked EPSCs by blocking the inactivation of action potentials in presynaptic terminals as it has been shown for Ia-afferents in cat spinal cord (J. Neurophysiol. 62: 1461, 1989).

420.1
ADDITION OF NERVE OR NERVE CONDITIONED MEDIUM TO CULTURED XENOPUS MYOCYTES ALTERS ACETYLCHOLINE RECEPTOR CHANNEL POPULATION AND KINETICS

J. Rohrschneider and Y. Kidokoro. Dept. of Physiology, U.C.L.A. School of Medicine, Los Angeles CA, 90024.

Single-channel recordings of acetylcholine receptor (AChR) channels were taken to assess changes in population and kinetics after the addition of nerve or nerve-conditioned medium to Xenopus myocytes cultured alone for several days. Muscle cultures were taken from stage 16-18 embryos, and stage 22-23 neural tubes or nerve-conditioned medium was added after 4-5 days in culture. It was previously found that the addition of nerve to Xenopus myocyte cultures had the surprising effect of increasing mean AChR channel open time in nerve analysis (Brehm et al. Dev. Biol. 91, 1982), indicating an increase in the relative number of low conductance (low-g) channels. We verified this effect, measuring the percentage of high conductance (high-g) single channel events after 6 days in culture. In control 6-day cultures, the percentage of high-g events was 54%. The percentage decreased to 30% and 27% when neural tube cells were added on day 3 or day 4, respectively. The effect was similar in both nerve contacted and non-contacted cells. Similarly, after one day exposure to nerve-conditioned medium, the percentage of high-g events decreased to 39%. This result suggests the possible release of factor(s) by the nerve which alters the synthesis rate of the low-g receptor. A remaining question is whether the kinetics of either the existing or of the new receptor population are altered after exposure to nerve. In anecurally cultured Xenopus myocytes, channel burst duration, particularly for the low-g channels, was significantly decreased. After 6 days, most low-g burst duration histograms have an excess of brief events but are otherwise fit with a single exponential. Preliminary results suggest that in many cases, low-g burst duration histograms are markedly double exponential, with a larger slow component, after nerve is added.

420.2

The role of the epsilon subunit in determining ACh receptor channel function was tested by co-expressing rat epsilon RNA with various combinations of mouse α2, and 8 RNA. Injection of α2 RNA into Xenopus oocytes resulted in several conductance classes, bearing long channel open time whereas injection of 8 RNA led to multiple channel types all bearing brief open time. We tested the possibility that the epsilon subunit-containing receptors were more sensitive to differences in subunit composition by injecting specific combinations of subunit RNAs. In the combinations of two subunits tested with ε1M ACH, only showed reasonable expression with α2, 8 and α4 expressing little or no current. However, all of the combinations containing 3 subunits tested, α2, 8, α2, and α4 expressed functional receptors, with α4 exhibiting the largest currents (1-10μA). Single channel recordings from α4 channels revealed a single amplitude class. The events corresponded to the intermediate conductance class observed with the full complement of α4 on the basis of similarities in both open time and conductance. Our findings indicate that inclusion of a subunit in the formation of functional receptors results in brief open times characteristic of receptors on adult skeletal muscle. The finding that the epsilon confers brief open times to ACh receptors of different subunit composition suggests that this subunit differs from α, β, and γ in that it plays an active role in shortening channel open time of ACh receptors. (Supported by NIH grant 18205 to PB).

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
420.3
NITRICOTINIC ACETYLCHOLINE RECEPTORS OF INSECTS AND VERTEBRATES: ACTIONS OF A BISQUATERNARY AMMONIUM SERIES.
C.A. Leech*, S.C.R. Lunniss*, C.W. Hoylake and D.B. Satelle
AFRC Laboratory, Macleod Site, University Department of Zoology, Cambridge CB2 3EJ, U.K. and E.I. du Pont Agricultural Products Department, Wilmington, DE 19880
Patch-clamp studies on dissociated CNS neurons of housefly (Musca domestica) and cockroach (Periplaneta americana) have revealed 3 distinct conductances in the presence of acetylcholine (20µM; 30-40pS; 60-80pS), of which the 30-40pS channel is the most open. Times are best fitted by 2 exponential components. Closed time distributions show greater variation and require 2-4 exponential fits. Voltage-clamp studies on identified cockroach neurons have characterized a nicotinic receptor blocks by a-bungarotoxin, dihydro-beta-erythroidine and loxototoxin. Displacement of [125I]a-bungarotoxin binding by a C4-C12 bisquaternary ammonium series of compounds shows that the cockroach CNS, chick CNS and chick muscle respond differently to an increase in molecular size of these compounds. Whereas in chick muscle, the percent inhibition increases with separation of the quaternary ammonium, brain inhibition is greatest at the two extremes. The cockroach receptor exhibits some of the properties of both classes of vertebrate nicotinic receptors in its response to the C4-C12 compounds.

420.5
BOTH ALPHA AND BETA SUBUNITS CONTRIBUTE TO THE AGONIST SENSITIVITY OF NEURONAL NITRICOTINIC ACETYLCHOLINE RECEPTORS.
C. W. Latue and J. Patrick, Division of Neuroscience, Baylor College of Medicine, Houston TX 77030
A family of genes has been identified which encode subunits of nicotinic acetylcholine receptors (nAChR), which are expressed in the nervous system. The α2, α3, and α4 subunits can each form functional nAChR when expressed in Xenopus oocytes in pairwise combination with either the β2 or β4 subunits. We find that the agonist pharmacology of mouse muscle nAChR (α1β1ε) expressed in oocytes matches that of the nAChR expressing mouse muscle cell line BCH11, demonstrating the accuracy of the oocyte expression system in the ligand gated ion channel pharmacology. Each of six neuronal subunit combinations displays an unique rank order of sensitivity to four nicotinic agonists. The α2β2 combination is 3-10 fold more sensitive to nicotine than to acetylcholine, while the α2β3 combination is 10-30 fold less sensitive to nicotine than to acetylcholine and the α3β4 combination is equally sensitive to both nicotine and acetylcholine. nAChR composed of α2, α3 or α4 in combination with β2 are 10-100 fold less sensitive to cytisine than to acetylcholine. In contrast, nAChR composed of α2, α3 or α4 in combination with β4 are 3-10 fold more sensitive to cytisine than to acetylcholine. The α2β2, α3β2 and α4β2 combinations are each equally sensitive to dimethylphenylpyridinium (DMPF) and acetylcholine, while the α2β4, α3β4 and α4β4 combinations are 3-10 fold less sensitive to DMPF than to acetylcholine. Our results demonstrate that the sensitivity of neuronal nAChR to various agonists is dependent not only upon which a subunit, but also upon which β subunit forms the receptor.

420.6
Molecular interactions of ethanol (EtOH) with a transmitter—gated ion channel were studied. In this work, we examined the concentration-dependence of EtOH actions on the kinetics of muscle nicotinic acetylcholine receptor (AcChR). Single channel currents were recorded from the peripheral region of isolated frog muscle fibers under cell-attached patch-clamp. We tested a wide range of EtOH concentration (0—1.7 M) on the current evoked by acetylcholine (ACH, 0.4 M). Many distinct kinetic alterations that showed concentration—time— and voltage—dependence could be depicted (fig 1). Changes in currents tested, the mean number of block per burst was increased; this effect was enhanced by hyperpolarization and was further accentuated by a prolonged drug exposure (fig 2). EtOH elicited two forms of block: a time—dependent and voltage —dependent one. When given current (IC) which grew linearly with hyperpolarisation (~ 10 to ~ 100 mV). At positive membrane potentials, the amplitude of the currents was smaller indicating the occurrence of anion (α) EtOH (1.7 M) decreased the channel conductance and induced two kinetically distinct populations of currents—one comprised of rapid events, and another consisting of long—lasting bursts. In addition, EtOH alone (0.6—1.7 M) activated currents that resembled those seen in the presence of both ACh and 1.7 M EtOH. Each compound of multiple action of EtOH on protein and/or lipid components of postjunctional membrane. Support: Capes/Finep/UFRJ—UAMAB Mol. Pharmacol. Training Program, and NIH #P01GM44211.

420.7
NITRICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) ION CURRENT CHANNELS IN RAT HIPPOCAMPAL NEURONS. M. Alkondon and E.X. Albusqeurque. Dept. Pharmacol. & Exp. Ther., Univ. of Maryland School of Medicine, Baltimore, MD 21201.
Mapping experiments using radiolabeled choline agonists and in vivo studies using extracellular recordings indicated that nAChR are present in different parts of the brain. Single—channel studies from our laboratory (Aracava et al., 1990, 272-283, 1990) showed the existence of functional nAChR channels in cultured rat hippocampal neurons. Very recently, a cationic channel for nAChR has also been shown in cultured neurons. This channel is found to be sensitive to the inhibition of nicotinic acetylcholine receptors by nicotinic receptors of the nucleus of the rat and from porcine hypophysial intermedia lobe cells. This study characterizes the nAChR in hippocampal neurons using whole—cell patch clamp techniques. Hippocampal cell firing rates grows in culture for 3—8 weeks were used and agonists were applied to the neurons using a U1—tube perfusion system. Acetylcholine (ACH) (50—100 µM) and (-)nicotine (Antx) (10 µM) inhibited the current (IC) which grew linearly with hyperpolarisation (~ 10 to ~ 100 mV). At positive membrane potentials, the amplitude of the currents was smaller indicating the occurrence of anion (α) EtOH (1.7 M) decreased the channel conductance and induced two kinetically distinct populations of currents—one comprised of rapid events, and another consisting of long—lasting bursts. In addition, EtOH alone (0.6—1.7 M) activated currents that resembled those seen in the presence of both ACh and 1.7 M EtOH. Each compound of multiple action of EtOH on protein and/or lipid components of postjunctional membrane. Support: Capes/Finep/UFRJ—UAMAB Mol. Pharmacol. Training Program, and NIH #P01GM44211.
Inhibition of Excitatory Amino Acid Currents by General Anesthetic Agents. Robert W. Peoples, David M. Lovinger, and Forrest F. Weight. Section of Electrophysiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

Previous studies in this laboratory have shown that ethanol and other aliphatic alcohols inhibit responses mediated by N-methyl-D-aspartate (NMDA) receptors, but not by physiologically relevant concentrations. We have investigated the actions of various anesthetic agents on excitatory amino acid-activated ion currents (IC50 - 50 µM) and on glutamate-activated ion currents in neurons using the whole-cell patch-clamp technique. Pentobarbital inhibited kainate and quisqualate currents with similar potency (IC50 - 50 µM) but produced a greater degree of inhibition of the quisqualate current (70% inhibition vs. 55% for kainate). Pentobarbital blocked both kainate and quisqualate currents in a use-dependent but rapidly reversible manner, and thus may bind to a site within, or near the opening of, the ion channels. Pentobarbital had essentially no effect on the NMDA current. In contrast, diethyl ether exhibited a pharmacological profile similar to that of ethanol, in that it inhibited the NMDA current to a greater extent than the kainate and quisqualate currents (~ 80% for NMDA vs. ~25% for kainate at 80 nM diethyl ether). We are currently investigating the effects of other anesthetic agents on excitatory amino acid currents. Inhibition of excitatory amino acid neurotransmission in the CNS by anesthetics could be expected to contribute to their depressant effects on behavior. (R.W.P. is a fellow of the National Research Council.)
420.16
LOWER CONCENTRATIONS OF KAINATE ARE REQUIRED TO ACTIVATE LOW CONDUCTANCE (2pS) KAINATE CHANNELS THAN HIGH CONDUCTANCE (20pS) ONES. L. M. Nowak and J. L. Chapman*, Dept. of Pharmacology, NYSCCM at Cornell University, Ithaca, N.Y. 14853.
Ascher and Nowak (1988, J.Physiol.299: 227) previously reported that kainate application (50-100pM) activates two populations of ion channels in outside-out patches from mammalian neurons, one with an estimated conductance of 2pS and open time of 3-4ms, and the other with an estimated conductance of 20pS and open time of <1ms. The conductances could be observed separately in different patches and it was suggested they may be associated with different kainate receptors; however, both responses were observed together in most patches.
Recently, we studied 2pS channels and determined that the 2pS channel is activated by lower doses of kainate (5pM) than the 20pS channel (>40 pM) in the same patches at ~60V. Recordings were performed on outside-out patches from cultured mouse brain neurons. The bath solution contained 300 mM NaCl and (in mM): 150 NaCl, 2.8 KCl, 1 CaCl2, 10 Hepes-Na (pH 7.2). The pipette solution contained (in mM): 140 CsCl, 2 Hepes-K (pH 7.2), and 10 EGTA/1Ca. Kainate (5-100uM) was applied in the bath solution by direct superfusion from a Pasteur pipet. 20-50 pA kainate evoked relatively large inward currents accompanied by a small increase in noise. Analysis of the variance in the noise indicated activation of a 2pS channel. Power spectral density analysis suggested the channel open time (t) was near 10ms. Application of 80-100m kainate to these patches gave an estimated channel conductance of 5-6pS from variance analysis and power spectra fitted by two Lorentzians, one a very high frequency component (<1ms), and the other one about 3-4ms. Thus, the shift in the aggregate conductance from about 2pS to 7-8pS suggested that increasing the kainate dose recruited the higher conductance (20pS) channels. This idea was supported by changes in the power spectra, which began to show a second high frequency component that contributed proportionally more to the total power as kainate concentration was increased. In the recruitment, the lower frequency peaks t (t = 10ms) was obscured and estimated open time approached 3-4ms >50pM kainate.

420.17
Glutamate depolarizes motoneurons in adult spinal cord. It is unclear, however, whether glutamate-sensitive channel characteristics seen in cultured embryonic cells are also present in adult animals. Therefore, membrane currents in response to glutamate application of glutamate agonists were examined in acutely dissociated spinal cord cells from adult rats using outside-out and whole-cell recording techniques. 400-um slices were cut from rat lumbar spinal cords, and 1-mm punches of gray matter were removed from the ventral horns. Following incubation in tissue, cells were dissociated mechanically and plated for electrophysiological analysis. Action potentials could be induced by depolarization in cells with resting potentials of approximately ~60 mV. Desensitizing 27-pS NMDA-sensitive channels were recorded; conductance was voltage-dependent, and addition of 1-mM glycine increased whole-cell currents. Likewise, desensitizing 10-pS quisqualate-sensitive channels and nondesensitizing 12-pS kainate-sensitive channels were observed. Supported by NIH grants NS1650 and F2B 220.
431.3  

We have previously reported that n-ethanol enhances GABA-induced current before desensitization occurs (Nakahito et al., PASEB J. 4, Al201, 1990). Ethanol and n-octanol have now been found to modulate the desensitization process of GABA receptor-chloride channel complex. The whole-cell patch-clamp technique was used to record currents from the rat dorsal root ganglion neurons in primary culture. The application of 0.3 μM GABA generated a peak current that decayed to a steady-state level. The initial phase of current decay was fit by a single exponential function, and the time constant was measured. The time constant of recovery from desensitization was also determined by paired applications of 0.3 μM GABA at various intervals. Ethanol (0.3-1 M) and n-octanol (30-100 μM) decreased the time constants of both phases in a dose-dependent manner. Neither ethanol (0.3 M) nor n-octanol (1-3 M) caused significant suppression of the desensitized current. The results indicate that alcohols modulate GABAergic neurotransmission in a phasic and tonic manner. Supported by ADAMHA grant AA07836.

431.5  
MODULATION OF TRANSMITTER-ACTIVATED MEMBRANE CURRENTS IN CULTURED SPINAL CORD NEURONS BY VARIOUS ADAMANTANE DERIVATIVES. H. Lampe*, H. Bigalke* (SPON: Europ. Neurol. Ass.), Department of Pharmacology and Toxicology, Med. Sch. of Greifswald, 17400 Hanover 61, FRG.

The agents 1-adamantanemethanol and 3-adamantan-3-ylmorpholine have been used in the treatment of M. Parkinson and movement disorders. In contrast to 1-adamantanemethanol and 3-adamantanemethanol and two other adamantane derivatives, 1-adamantanecarboxyaldehyde and 1-adamantanemethylcarbinol suppress picrotoxin-induced hyperactivity in cultured neurons, which indicates also an anticonvulsant potential (Netzer, R. and Bigalke, H. Europ. J. Pharmacol., in press). To elucidate the underlying mechanisms, effects of the drugs on neurotransmitter-mediated currents were investigated. We used the whole-cell patch clamp technique to record glutamate- and glutamate-activated whole-cell currents. Cultures were continuously superfused with growth medium containing 4 mM Mg2+. Patch pipettes were filled with low calcium/high potassium saline. Either glutamate or glycine were applied by pressure ejection to cells clamped at -50 mV membrane potential. Each of the drugs reduced the glutamate-activated current in a concentration-dependent fashion, except for 1-adamanatane which had no effect. The glutamate receptor-mediated current was promoted by 1-adamantanemethylcarbinol at concentrations of between 0.1 μM and 5 μM, but was reduced at higher concentrations. 1-Adamantanecarboxyaldehyde and 1-adamantanemethylcarbinol antagonized the glutamate receptor-mediated current in a concentration-dependent manner. No effect was observed with 3-adamantanemethylcarbinol. Supported by ADAMHA grant AA07836.

431.6  
EFFECTS OF DELTA-9-THETRAYUCANABINOL ON WHOLE-CELL CURRENTS IN CULTURED RAT HIPPOCAMPAL NEURONS. Robert E. Hampson, Shao Wang, Barbara A. Bennett, Mariana Morris and Sam A. Dargan, Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina 27103.

Studies in this laboratory have demonstrated an effect of delta-9-tetrahydrocannabinol (THC), the psychoactive ingredient of marijuana, on hippocampal neural activity (Hampson et al., Neurosci. Abstr., 15:1170, 1989; Campbell et al., JPEP, 239:941-945, 1986) in awake, freely-moving rats. A recent report by Herkenham et al. (PNAS, 87:1932, 1990) demonstrated binding of a radio-labeled THC agonist (CP-55,940 - P40) to a high-affinity THC receptor in rat hippocampus. To examine THC-receptor interactions, we applied whole-cell patch clamp techniques to primary cultures of dissociated hippocampal neurons. Voltage-clamp recordings from cells in culture for 5-10 days, using KCl-filled patch-clamp pipettes showed an inward membrane current at resting potential following preapplication of 1 μM to 30 μM THC to the outside of hippocampal neurons. The inward current was increased at hyperpolarizing potentials and reversed at depolarizing membrane potentials. Reversal of this current was consistent with the equilibrium potential for chloride using high chloride concentrations in the patch pipettes, and was mimicked by application of GABA in concentrations similar to those of THC. Substitution of acetate for chloride in the recording pipette produced outward currents. Substitution of chloride-free bathing medium reduced or eliminated the inward current. Bath application of THC also reduced voltage-sensitive sodium and potassium currents. Comparison of THC with other THC analogs for ligand-gated membrane currents will be determined.

[Supported by grants DA04019, DA03502 & DA04441 to S.A.D., DA05073 to R.B.E. and NS22492 to M.M.]
ION CHANNELS: LIGAND-GATED II

421.9
ENDOTHELIN-1 PRODUCES MEMBRANE DEPOLARIZATION ACTIVATING CALCIUM AND CHLORIDE CURRENTS THROUGH CYCLIC GMP DEPENDENT SYSTEM. T. Kishimura†, S. Akasu*, and J. Krieger†
The ionic mechanism(s) underlying endothelin-1 (ET)-induced membrane depolarization of guinea pig vas deferens was studied. Membrane currents in rabbit vagal pelvic ganglia were recorded in vitro using single electrode voltage clamp. Cells were isolated by electrodes filled with 2 M CsF in a modified Krebs solution (36°C) containing 50 mM TEA, 2 mM CaCl2 and 300 mM TTX. At a holding potential of -60 mV ET ([0.1-2 μM] current (I1a; 0.2-2.6 nA) followed by an outward current (Iout; 0-1.0 nA). I1 in and Iout were associated with increased and decreased membrane conductance respectively. In CaF2 solution I1 was reduced to 25.8±3.9% of control, while Iout was blocked. Ca-Inensitive I1 was blocked by 4-acetamido-4-isothiocyanostilbene-2,2-dilphonic acid (0.5 μM; chloride channel blocker). Reversal potential of I1, estimated by current-voltage relationship, was +42±3 mV (n=4) and -18±2 mV in normal CaCl2 (1.25 mM) and CaF2 solution, respectively. Effects of ET were mimicked by application of dibutyryl guanosine 3,5′cyclic monophosphate (db-cyclic GMP; 10-200 μM). Pretreatment of db-cyclic GMP (10 nM for 10 min) blocked endothelin-induced current responses. We conclude that ET activates both CaCl2 and CaF2 conductances through a cyclic GMP-dependent signal transduction system.

421.10
ON THE POTASSIUM CONDUCTANCE INCREASE ACTIVATED BY DOPAMINE IN THE JELLYFISH, Polyorchis penicillatus. J. M. Chung and A. N. Spencer. Dept. of Zoology, Univ. of Alberta, Edmonton, Alta, Canada, T6G 2E9
The effects of dopamine (DA) applied by plicoting onto cultured swimming motor neurons of Polyorchis penicillatus were examined using whole-cell recording technique. DA caused hyperpolarization and a decrease in the firing frequency. The effect of DA was accompanied by a fall in input resistance. Under voltage clamp, DA produced outward currents associated with a conductance increase and showed desensitization with prolonged agonist applications. The DA effects were concentration dependent (effective range 10 nM to 100 μM). The DA currents reversed polarity around -55 mV in the negative conductance potentia.

422.1
PEPTIDES: PHYSIOLOGICAL EFFECTS III

422.1
EFFECTS OF A NEW TRH ANALOGUE, YM-14673 ON NEUROLOGICAL DEFICITS IN RATS SUBJECTED TO BOTH INTERNAL CAPSULE LESION AND MOTOR EXERCISE. M. Kansamata*, S. Kawakita*, S. Yatsuga*, A. Iwii†, S. Kawabata, F. Wanibuchi, and M. Yamamoto†
Central Res. Labs., Yamaneuchi Pharmaceutical Co. LTD., Tsukuba, Ibaraki 305, Japan
Effects of YM-14673, a potent and long lasting new TRH analogue were studied in rats subjected to lesion of the left internal capsule(1C) under the motor exercised condition. In this model, neurological deficits such as hemiplegia, and decrease in amplitude of EMG activity evoked by electrical stimulation of the left sensory motor cortex were observed on the right legs. Both motor exercise using swimming, treadmill or wheeling drum techniques, and drug administration started from 4 days after surgical operation and repeated once a day for 10-16 days. Motor exercise using wheeling drum technique accelerated the recovery of neurological deficits in IC rats both with and without motor exercise. These results suggest that the beneficial pharmacological properties of YM-14673 encourage the therapy in stroke patients with rehabilitation.

422.3
Galanin and Hypotropon-releasing hormone (TRH) were shown to be present in superficial laminae of the dorsal horn (DH), but the data regarding their physiological function are not available. In order to investigate the effect of these two peptides we employed a technique of intracellular recording from DH neurons in an in vitro spinal cord slice preparation. The preparation consisting of a rat spinal cord DH slice (300-500 μm thick) with intact dorsal roots was stimulated from single DH neuron was performed with 3M K-acetate back-filled glass microelectrodes having DC resistance of 100-150 MΩ. Dorsal roots were stimulated by using bipolar platinum stimulating electrodes. The threshold of action potentials in DRG neurons and have been found to be 8-20V/0.2ms for the most sensitive fibers. In superficial dorsal horn (DH) neurons, the threshold of galanin (10-7 M to 10-4M) produced a dose dependent depolarization in about half of the neurons tested (n=15). The depolarization was associated with an increase in amplitude of action potential, while the firing rate was unaffected. Furthermore, the amplitude of a prolonged offset action potential in some neurons (+5). In addition to increase in spontaneous firing, galanin had a facilitatory effect on synaptic activation evoked by dorsal root stimulation at intensities sufficient to activate myelinated and unmyelinated afferents. TRH produced depolarization was associated with increase in conductance and firing of action potentials. Work was supported by NIH grant NS27751 and USDA grant PL95-113.

422.4
MORPHINE INDUCES THE MODULATED RELEASE OF POMF-NH2-LIKE PEPTIDE FROM RAT SPINAL CORD. J.M. Zhu and H.-Y.T. Yang, Lab. of Biochem, Genetics, NIMH, Neuroscience Center at St. Elisabeth, Washington, DC 20032
Several lines of evidence indicate that POMF-NH2-like peptide, FMRF-NH2-like peptide from the brain, may have a role in opioid mediated antinociception. In rat spinal cords, high density of F-8-F-NH2 immunoreactivity was detected in superficial lamina of dorsal horn and the presence of specific P-8-F-NH2 receptors was also demonstrated. These studies suggest a possible function as neurotransmitter or neuropeptidergic in the spinal cords and effect of morphine on this secretion were studied by an in vitro superfusion procedure. The spontaneous release of P-8-F-NH2 was high at the begining of the superfusion and then gradually decreased to a steady level of 7 fmol/mL in calcium dependent manner. DC in the perfusion medium was increased to 55 μM. The released F-8-F-NH2 immunoreactivity was analyzed by HPLC. F-8-F-NH2 was followed by RIA and the main immunoreactivity released was found to be identical to the main immunoreactivity detected in the spinal cord extract. Addition of morphine to the perfusion medium was found to inhibit the 56 μM KC induced release of F-8-F-NH2, in a dose dependent manner. Thus the results suggest with the hypothesis that F-8-F-NH2 may participate in opioid mediated antinociception.
422.5 SUBTYPES OF GLABINE NUCLEOTIDE-BINDING REGULATORY PROTEINS INVOLVED IN THE SUPPRESSION OF BARORECEPTOR REFLEX BY NEUROTENSIN IN THE RAT. Samuel H. Chen, J. M. Fox, B. K. Ling, and Cheryl Tang-Ning Red, Goli, and Taipei Veterans General Hospital, Taipei, Taiwan, ROC. We attempted to identify the subtypes of glabine nucleotide-binding regulatory proteins (G-proteins) that may underlie the suppressive effect of neurotensin (NT) on the baroreceptor reflex (BRR), using Sprague-Dawley rats that were anesthetized with pentobarbital sodium. Intracerebroventricular (i.c.v.) application of NT (15 ng) significantly inhibited the BRR response. Such an inhibition was appreciated with GABAergic, excitatory, and parasympathetic responses, and the effects of NT were not modified by application of 5-HT3 antagonists, H-1 antagonists, or a-adrenergic antagonists. These results suggest that NT may act by modulating the release of neuropeptides (NTS) to the nucleus tractus solitarius. Supported by the Medical Research Council of Canada.

422.6 EXCITATORY ACTION OF SOMATOSTATIN (SST) ON RAT AMBIGUAL MOTONEURONS IN VITRO. Y.T. Wang, R.S. Neuman & D. Bieger. Faculty of Medicine, Memorial University of Newfoundland, St. John’s, Newfoundland, Canada, A1B 3V6.

The occurrence of SST in subnuclear centralis (NTS) neurons of the solitary complex has recently been reported. NTS neurons send a dense projection to the compact osphagophasalubovascularization of the ambiguous complex (AMB). Using intracellular recording with KCl (3M) or K-methyl-SO4 (2M) filled microelectrodes from sagittal brainstem slices maintained at 37°C in a 95% O2-5% CO2 atmosphere of saline, we have investigated the effects of SST on AMB neurons. Pneumorphometric application of SST induced a) an immediate dose-dependent membrane depolarization with associated spiking, b) little or no change in membrane conductance, and c) an inward current recorded under voltage-clamp in the presence of TTX. There was no change in post-spike hyperpolarization and no evidence of desensitization with test intervals of 2 min. We conclude that SST is a prime candidate for excitatory transmitter in the NTS-AMB pathway that and its role in osphagophasal peristalsis merits further studying.

Supported by MRC (Canada)

422.7 RESPONSE OF NEURONS IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS TO THYROTROPIN-REleasing HORMONE (TRH). C.A. Livingstone and A.J. Bieger. Dept. of Physiology & Biophysics, Univ. of Washington School of Medicine, Seattle, WA 98195.

Preganglionic parasympathetic neurons of the dorsal motor nucleus of the vagus (DMX) regulate visceral motor activity. Using an in vitro slice preparation of the guinea pig brainstem, we determined the effects of TRH (one of numerous neuropeptides localized within the DMX) on DMX neurons. Whole-cell (30-μm thick) from celiac medulla were superfused with standard Ringer's solution, and the responses of DMX neurons to bath-applied TRH were recorded in current clamp conditions. In all cells tested, TRH (3-10 μM) induced a reversible 2-18 mV (9, n=7) depolarization by a decrease in membrane conductance, which persisted over many minutes. A similar response was evoked in the presence of low Ca²⁺, high Mg²⁺ (Ca²⁺ spikes and Ca²⁺-dependent K⁺ currents abolished), indicating that TRH acts directly on these cells. TRH applied in the presence of 0.5 mM TTX or 0.5 mM TTX and 2 mM CsCl similarly depolarized these cells, suggesting that the response is not mediated exclusively via TTX-sensitive current. Evaluation of the I/V relations of the cells in the presence and absence of TTX indicated the reversal potential of the response to be -75 to -90 mV. We suggest that the TRH-induced depolarization of DMX neurons is due to a reduction of an outward cationic current. (Supported by NS144857 and a Parker B. Francis Foundation Fellowship)

422.8 ANGIOTENSIN II AND SUBSTANCE P EXCITE NEURONS IN THE RAT MEDIAL NUCLEUS TRACTUS SOLITARIUS (mTS) IN VITRO. K. L. Barnes, W. D. Knowles and C. M. Ferrario. Departments of Brain and Vascular Research and Neurology, Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195.

There is a striking congruence between the patterns of high affinity binding sites and neuronal elements immunoreactive for angiotensin II (Ang II) and substance P (SP) in the rat mTS. Although both peptides influence autonomic function via this region, Ang II is thought to subserve cardiovascular function, while SP appears to exert broader sensory and autonomic effects. To examine how peptide effects act on the same or different neurons, we compared the effects of Ang II and SP on mTS neurons recorded from in vitro slices of the rat medulla. Horizontal slices (400 μm) containing the mTS were perfused with artificial cerebrospinal fluid (aCSF, 36°C). Extracellular recordings were obtained from 20 mTS neurons during microapplication of Ang II or SP (both 1 μM in aCSF) onto the slice surface. Ang II substantially increased the firing rate of 8 of the 20 cells tested, while SP excited 10 of 14 cells given this peptide. The time course of the response to SP was similar to that for Ang II. No inhibitory effects were seen with either peptide. In 14 neurons the effects of Ang II and SP were compared. Three cells responded only to Ang II, 6 neurons were excited only by SP, 4 cells were excited by both peptides, and 1 neuron did not respond to either peptide. These studies reveal a partial overlap of the neuronal substrate that mediates the autonomic effects of Ang II and SP via the mTS, and suggest that there may be interactions between Ang II and SP in certain pathways of the mTS. (Supported in part by NHLBI grant HL-6835).

422.9 ANGIOTENSIN II INHIBITS GLUTAMATE-ERIVOK DEPOLARIZATION OF LOCUS COERULEUS NEURONS IN VITRO. Huangxi Xiong and Kenneth G. Marshall. Dept. of Physiology, University of Ottawa, 651 Smyth Road, Canada K1H 8K5.

The effects of iontophoretically applied angiotensin II (AIIT) on intracellularly recorded locus coeruleus (LC) neurons were studied in transverse pontine slices from rats weighing 50-100 g. The major effect of AIIT was a specific depression of the depolarization and resultant excitatory produced by iontophoretic glutamate (Glu) application (39/47 neurons). Application of Na+ ions with equal or higher currents had no effect on membrane potential or responses to iontophoretic Glu. The action of AIIT was accompanied by a small hyperpolarization of the cell membrane potential in a few neurons. However, in most cells, the depression of Glu actions occurred in the absence of changes in membrane potential, or amplitude, duration or shape of the action potential. AIIT had no effect on action potentials evoked by injection of depolarizing current, or by application of acetylcholine and thus appears to specifically inhibit Glu actions. The effects of AIIT on Glu excitations were antagonized by superfusion with low Ca²⁺/high Mg²⁺ (0.5 mM/10 mM) solution, indicating that a post-synaptic site of action of AIIT. The AIIT effects were not changed by superfusion with low Ca²⁺/high Mg²⁺ (0.5 mM/10 mM) solution, indicating that AIIT can selectively activate and potentiate inhibitory modulation of the post synaptic actions of glutamate. Supported by the Medical Research Council of Canada.

422.10 NEUROPEPTIDE Y (NPY) INHIBITS α-ADRENERGIC AND 5HT4- MEDIATED SYNAPTIC POTENTIALS IN DORSAL RAPHE IN VITRO. Samuel B. Kombian and William P. Colmers. Dept. of Pharmacology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Because the (entirely extrinsic) noradrenergic innervation of the largely serotonergic dorsal raphe nucleus also expresses NPY, receptors for this peptide are found there, and NPY has been shown to inhibit transmitter release in several tissues, we examined the effects of NPY on synaptic potentials in dorsal raphe. Intracellular recordings were made with 500 pM filled microelectrodes from rat dorsal raphe nucleus neurons in submerged in vitro slices. Focal electrical stimuli from bipolar tungsten electrodes placed in the nucleus were used to evoke synaptic potentials. Fast adrenergic (α1) and GABAergic potentials were succeeded by a slower, 5-HT4, autoreceptor-mediated IPSP and an even slower, α1-receptor-mediated EPSP. The slow EPSP evoked by focal stimuli averaged 3.9±0.6 mV, and peaked at 1833 ± 500 μS after the stimulus. This EPSP was completely eliminated by bath application of prazosin (100μM). Bath application of 1μM NPY caused a decrease in amplitude of the slow EPSP by 453±66% (P<0.01) of which reversed fully when washed out of the slice. The slow, 5-HT4 autoreceptor-mediated EPSP was decreased by peak of NPY from 129.9 ± 1.1 mV to 60.5 ± 1.1 mV, a decrease of 54±4% (7, P<0.05). There was no significant change in the amplitudes of the fast synaptic potentials. NPY did not alter the resting membrane potential or input resistance of these neurons. The results indicate that NPY may act to decrease the electrically-evoked release of noradrenaline from terminals in the dorsal raphe nucleus. Moreover, NPY appears to have an inhibitory effect on release of 5-HT from neurons within the dorsal raphe. The site and mechanism of NPY's actions in the dorsal raphe nucleus remain to be elucidated.

Supported by MRC (Canada). SBK is an MRC Student. WPC is a Medical Scholar of the Alberta Heritage Foundation for Medical Research.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
PRESYNAPTIC INHIBITION BY NEUROPEPTIDE Y (NPY) AND ADENOSINE IN RAT HIPPOCAMPUS IS BY A DIFFERENT MECHANISM THAN BACLOFEN. William P. Colmers and Gloria J. Klups, Dept. of Pharmacology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2G7.

NPY presynaptically inhibits excitatory transmission between stratum radiatum and CA1 principal neurons. This effect can be prevented by 4-aminopyridine (4-AP); however, reducing extracellular calcium restores NPY's inhibition, suggesting that it inhibits some, but not all, calcium channels in presynaptic terminals (Colmers, et al. J. Neurosci. 12: 671-679). Baclofen and adenosine A1 receptors mediates presynaptic inhibition at this synapse. We therefore examined whether these two receptors act via a mechanism similar to that postulated for NPY.

Population spontaneous extracellular electrical stimulation of stratum radiatum (75-90% of maximum response) were recorded with extracellular microelectrodes in the CA1 pyramidal cell layer of submersed transverse slices of rat hippocampus. Bath application of 1mM NPY, caused a reversible reduction of 91% in PS amplitude, which was reduced to only 26% in the presence of 4-AP, and recovered to 67% by 1.75mM extracellular Ca++. Although 4-AP caused a small reduction in the effect of 10mM baclofen, from 97% to 76%, low Ca++-4-AP had no significant effect. By contrast, no effect was seen with these treatments on the effect of 3mM 2-BA. To more carefully examine this, dose-response relationships for NPY, baclofen and 2-CA were constructed in saline, in 4-AP and low Ca++-4-AP. While 4-AP shifted the dose-response curve for NPY, 2-CA and baclofen rightward in a parallel manner, lowering Ca++ in the presence of 4-AP caused a parallel leftward shift only to NPY and 2-CA, while the 4-AP modulated rightward shift in the dose-response curve for baclofen was not shifted leftward by low Ca++-4-AP.

The results indicate that in hippocampal CA1, presynaptic NPY and adenosine A1 receptors may share similar mechanisms, while the presynaptic GABA_A receptor may act via different mechanism than the others. Supported by MRC (CANADA). WFC is an AHFSR Scholar.

CELLULAR EFFECTS OF ATRIAL Natriuretic PEPTIDE ON VASOPRESSIN NEURONS. K.M. Hurley and C.B. Soper, Deps. of Pharm. & Physiol. Sci. and Neurology, Univ. of Chicago, Chicago, IL 60637.

Atrial natriuretic peptide (ANP) is a potent natriuretic and diuretic hormone that also acts as a central neuromodulator. Systemically, ANP opposes the actions of arginine vasopressin (AVP). Intraventricular ANP administration secretions of AVP from the posterior pituitary gland. Application of ANP in femtomole doses to AVP neurons in the paraventricular nucleus increases the intervals between bursts of neuronal firing and shortens burst duration. Thus, the mechanism of this effect is not known. An indirect effect through magnocellular neurons in the suprachiasmatic and paraventricular nuclei in 350-400 µm slices through the rat hypothalamus. Micropipettes were filled with a 4% solution of biotin in 0.5 M KCl-0.03 M TRIS buffer. At the end of each experiment, cells were filled iohistopically with biotin and cell type was verified by combined FITC-immunofluorescence for AVP and Texas Red-streptavidin for biotin. Cells had an input resistance in the range of 130-160 MΩ, membrane potential of -60 to -80 mV and action potential amplitudes of 60-80 mV. Membrane conductance was monitored by 2Hz, 30 ms current pulses of -0.25A. ANP (0.01-10 fm) applied to the surface of the slice resulted in a decrease in membrane conductance and complex changes in membrane potential and cell firing pattern. Similar mechanisms may mediate AVP release in vivo.

The Nacc is of interest because of its suggested role in reward mechanisms and, possibly, opiate and cocaine abuse (Koob and Bloom, Science 242:715, 1988). This region contains abundant levels of opioid peptides, including enkephalins, dynorphins and endorphins. Therefore, we developed an in vitro slice preparation of Nacc and tested the effects of superfusion with opioid peptides on Nacc neurons. Intracellular recordings of these neurons revealed that many have unusual membrane properties, including: 1) large resting membrane potentials (avg. -81 mV; range: 48 to -97 mV; 24 neurons); 2) anomalous rectification, resulting in a 'lag' of the electrotonic potential generated by hyperpolarizing current steps; and 3) a time-dependent 'ramping' response to depolarizing current steps, resembling the effect of the D-current (Storm, Nature 336:379, 1988) in hippocampal neurons. However, other cells show a more linear I-V curve, suggesting the presence of more than one Nacc neuron type. Input resistances varied 51 MΩ. We found little effect of superfusion of several enkephalin analogues, DPen4, DPen5 enkephalin (a 6 opiate receptor agonist; n = 7), DAGO (a selective µ receptor agonist; n = 4) and D-Lys5-enkephalin (a broad spectrum µ, δ agonist; n = 1) on either membrane potential or input slope resistance, at 1-2 µM. However, all of these opioids dramatically reduced post-synaptic potentials (to 41% of controls; n = 6) evoked by afferent stimulation in the area ventral to the Nacc. These results suggest that the major role of opioid peptides in this region is to reduce synaptic transmission. (Supported by NIDA grant DA 05665.)


Last year we reported here small increases (100-205 over baseline) in IP3 release in rat hippocampal slices using 10 mM TRH in the presence or absence of lithium, 10 mM retinal is a GSH structure that is also known to contain TRH and its receptors. After a 1 hr. load-up with nyc-0-1 (i.e. as is noted above, a 20 min. chase with cold myo-inositol, and then a 15 min. preincubation in 10 mM Li+), individual retinal tissues were incubated for 30 min. more without and with 10 mM TRH. Reaction was stopped by freezing the tissues and quick-transfer holders, then extracting in chloroform-methanol. The IP3's were separated from de-chromatographically and total. DPM's were obtained from the chloroform fraction, tissue IP3's, tissue I and medium I. Results were expressed as percent of DPM in IP3's to total DPM's of all other fractions from each sample. Results cumulate the mean values. Basal IP3 in Long-Evans rats was 4.0 ± 0.9 (SEM) (n=13) vs. 5.5 ± 1.0 (n=13) with TRH (30 min. increase, P < 0.0019). Basal IP3 in Sprague-Dawley rats was 3.9 ± 0.8 (n=8) vs. 8.2 ± 1.8 (n=6) with TRH (110% increase, P < 0.0036). These may be the largest IP3 release effects of TRH so far reported in CNS, D-E curves and scanrrain will be pursued. Supported by VA Res. Serv.

ENDOGENOUS OPIOIDS RELEASED FROM PERFORANT PATH MODULATE NORPRENPHINE RELEASE AND IPSP AMPLITUDES IN GUINEA PIG CA3 PYRAMIDAL CELLS. M.M. Castell, J.E. Wagner*, C. Sacktor. Dept. of Pharmacology, University of Washington, Seattle, WA 98195.

Our previous work demonstrated that pharmacologic depolarization or electrical stimulation of opioid containing pathways in hippocampal slices could release endogenous opioids by releasing either 3-6 or 3-6-DAG binding. In this study we demonstrate that endogenous opioids were released by perforant path (PP) stimulation to affect synaptic responses recorded in CA3 pyramidal cells. CA3 pyramidal cells were impaled in perfused guinea pig hippocampal slices. Synaptic potentials in pyramidal cells were evoked by stimulation of the PP (100 µA, 0.033 Hz). A single stimulus train (10 µA, 10 Hz, 5 s) in the PP produced a small depression in IPSP conductance that lasted for approximately 15 min. The percent change when compared to unstimulated controls was -11.1 ± 8 (n=13, p<0.00). When naloxone (100 mM) was added to the bath and the PP was stimulated a large increase in conductance was observed (42.5 ± 27 % change, p<0.05, n=14). Following PP stimulation, the naloxone-induced change in IPSP conductance was delayed and prolonged; no effect was seen until 30 ± 6.6 min after stimulation; time to peak was 56 ± 0.9 min; total duration was 150 ± 19 min. The inhibition was specific to perforant path stimulation. Bath applied propranolol (1 µM, n=10) or pretreatment of the guinea pigs with reserpine (5 mg/kg, N=6) blocked the effect. These findings suggest that endogenously released enkephalin regulates the release of norepinephrine and that endogenously released enkephalin enhances IPSP conductance in the CA3 region of guinea pig hippocampus. Supported by DA04123.
DIFFERENT G PROTEINS MEDIATE THE OPIOID INHIBITION OR ENHANCEMENT OF EVOKED METHIONINE-ENKEPHALIN RELEASE. H. Xu and A.R. Gisclair. Department of Biochemistry, SUNY Health Sciences Center at Brooklyn, Brooklyn, NY 11203. This laboratory has previously demonstrated that there is an opioid receptor mediated enhanced inhibition of the electrophysiologically evoked release of methionine-enkephalin (enkephalin) from the guinea pig myenteric plexus. Low doses (nanomolar) enhance, whereas higher concentrations (10-100 nM) inhibit release. We now demonstrate that the islet-activating protein from porcine toxin (PTX; 50 µg i.p./500g for 6 days) can abolish the ability of mu, delta and kappa-selective opioids to inhibit the evoked release of enkephalin. In contrast, PTX is without effect on the enhancement of enkephalin release observed following treatment with nanomolar concentrations of the above opioids. Conversely, pretreatment with cholera toxin (CTX; 10^{-3}M for 3 hrs in vitro) has no effect on the mu, delta or kappa opioid inhibition of evoked enkephalin release but abolishes the ability of nanomolar concentrations of these agonists to enhance stimulated enkephalin release. These data further differentiate the signal transduction process responsible for each opioid effect. Furthermore, they suggest that a PTX-sensitive G protein (G_{o}, G_{i}) and a CTX-sensitive G protein (G_{q}) are integral components of the mechanism that mediates opioid inhibition and opioid enhancement, respectively, of evoked enkephalin release.

5 Different opioid peptides affect the secretion of vasopressin (VP) or oxytocin (OT) from the neonatal rat endocrine system, as well as from isolated pituitary cells. As a specific antagonist to the opiate receptor subtypes µ, κ and δ are now available, we examined the role of endogenous opioids in VP release from the hypothalamic region. Two hypothalamic regions of the brain were isolated: the neurohypophyseal hormone release became possible. Male Wistar rats (200 g), 24 hr water-deprived, were injected sc and decapitated. Plasma levels of VP and OT were determined by RIA in trunk blood. No effect on either hormone was found 30 min after application of the δ-agonist DPDPE (dose 0.01-5 mg/kg). However, the µ-agonist DALDA (Schilter 1989, J. Med. Chem. 32, 690) applied in the same dose range strongly inhibited the release of both VP and OT, an effect that was maximal 30-60 min after injection. The κ-agonist U50488 induced a similar effect: VP plasma levels even became undetectable. The time-response curve showed a maximal effect 30 min after application of the compound. Antagonism of endogenous opioids by the δ-agonist naltrindole (naltrindole 0.01-1 mg/kg) did not affect the release of either hormone, whereas both the δ-specific antagonist nor-binaltorphimine, and the relative µ-specific antagonist naloxone selectively enhanced OT plasma levels and not VP levels. This indicates a selective and endothelin inhibition of the OT release mediated by κ-agonistic opioids (e.g. dynorphins) although µ-agonistic opioids might be involved as well. Furthermore, the κ- and μ-opioid receptors seem to play an important role in regulating the secretion of both VP and OT from the neurohypophysis.

A NOVEL OPIOID CONTROL OF PROLACTIN SECRETION IN IMMATURE RATS. S. Blackford and C. Kuhl, Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710. The present study explores developmental changes in mu and kappa opioid receptor control of prolactin (PRL) secretion. The ontogeny of mu and kappa receptor function was determined by assessing the PRL response to the mu agonist sufentanil (1 mg/kg, s.c.) and the kappa agonist U50488 (1 mg/kg, s.c.) in 5, 10, 15 and 20 day old rats. Both mu and kappa agonists stimulated PRL secretion at all ages. Sufentanil produced a selective blocking of mu agonist-induced beta-farnesaximine and U50488 was selectively blocked by the kappa antagonist nor-binaltorphimine. Serotonin mediation of opiate-induced changes in prolactin secretion was explored in neonatal rats by testing cyproheptadine (CYP, 10 mg/kg) blockade of opiate responses in 5, 10, 20 and 60 day old rats. CYP provoked the PRL response to the mu agonist but did not affect the kappa agonist-induced changes in prolactin secretion. Serotonin blockade by hemin or 5,6-dihydroxytryptamine decreased mu agonist-induced changes in prolactin secretion. Serotonin blockade by hemin or 5,6-dihydroxytryptamine decreased mu agonist-induced changes in prolactin secretion.

ENDOCRINE REGULATION OF CELL PROLIFERATION IN THE DEVELOPING RAT RETINA, T. Isvanyi, P.J. McLaughlin, and J.S. Zagon, Penn. State Univ. Coll. Med., Hershey, PA 17033. The role of endogenous opioid systems in regulating cell proliferation in the developing retina was examined in 1-day-old rats. Met-enkephalin (MENK), naloxone (NTX), a mixture of MENK and naloxone (NALX) were administered to experimental animals; controls received sterile water. After three to four hour periods, animals were injected with 0.01 µg of H3-thymidine and sacrificed one-and-a-half hour later. The labeling index of DNA-synthesizing cells within the germinative layer (GL) of the developing retina was determined from autoradiographs. 15% less than control animals. MENK-treated retinas showed a 10% decrease in the proliferating cell population in response to MENK treatment. MENK-NALX-treated retinas exhibited no change. Immunocytochemical preparations of 1-day-old rat retina showed that MENK immunoreactivity was abundant in the ganglion cell layer (GCL) of the retina, but was absent in the inner nuclear layer (INL). These results suggest that opioids are present in the retina and act as natural inhibitory trophic factors to tonically govern cell replication in the developing eye.

Supported by NIH NS20500.
OPIOIDS:

**3.1**

Presence of Zeta (β), a Growth-Related Opioid Receptor, in Rat Cerebellum. I.-J. Zagon, D. Gibb, and P.J. McLaughlin. Penn State Univ. College of Medicine, Hershey, PA 17033.

Interaction between endogenous opioids and opioid receptors has been shown to regulate cell proliferation in the developing rat brain. [Met-]enkephalin (ME) is a very potent opioid antagonist which binds to one of a tripeptide sequence that inhibits cell replication. [D-Me]ME was used to probe the binding site of 6-day-old rat cerebellum. Specific, high affinity, and saturable binding of [D-Me]ME was recorded in cerebellar homogenates; Kd=2.0±0.3 nM, Bmax=23,117 fmol/mg protein. Binding isotherms were linear with protein and constant on time, prior to detergent or trypsin digestion. Addition of Na+, Mg++, and guanyl nucleotides reduced binding; trypsin eliminated binding, suggesting a protactinophilic binding site. Competition studies using a wide variety of endogenous and exogenous opioids, including those selected for other opioid receptor subtypes, revealed that ME was the most selective ligand for the binding site. These results indicate the presence of a new opioid binding site, zeta (β), in the developing nervous system which is related to cell proliferation. Supported by NIH grant NS50500.

**3.11**

EFFECTS OF U- AND K-AGONISTS ON THE INTRACELLULAR FREE CALCIUM IN ISOLATED RAT MYOCYTES. X.D. Huang*, K. Tsou and T.M. Wong*. Department of Physiology, University of Hong Kong, Hong Kong.

U-Wong et al. (unpublished) k-agonists (Wong and Lee, Neurosci Lett 77, 61-66, 1987) have been shown to induce arrhythmias in the isolated perfused rat heart. Since alterations in calcium fluxes are related to arrhythmias, we have studied the effects of these two agonists on intracellular free calcium (Ca2+) in isolated myocyte with a spectrophoto metric method using fura-2 AM as the florescence indicator. Isolated myocytes were prepared from hearts of female Sprague-Dawley rats of 100-150 g according to the method of Young and Scarpas (FAE Lett, 223,53-58, 1987). U-agonists, D-Ala2,MePe, Gly-ol-enkephalin (Peninsula lab.) and morphine and k-agonists, dynorphin (35) (Peninsula Lab) and U-47508 (Upjohn Co.), increased Ca2+. The effect was blocked by their respective antagonists, naloxone (DuPont Pharmaceutical Co.) and MR 2266 (Boehringer Ingelheim), which did not produce any effect themselves. Chronic injection with morphine at increasing doses to rats according to the procedure of Wong and Lee (Neurosci Lett 77, 61-66, 1987) or addition of morphine (100 μM) to cultured isolated myocytes at 12 h before experiment also abolished the effect of morphine in elevating Ca2+. The results of the present study showed that μ-k-agonists increase the Ca2+ via opioid receptors on the membrane of myocytes. Such effect is probably responsible for their arrhythmogenic action in the isolated perfused rat heart. (Supported by a Croucher Foundation Grant to T.M.W. and MR 2266, naloxone and U50,889H were kindly supplied by Boehringer Ingelheim Co., DuPont Pharmaceutical Co and Upjohn Co., respectively).

**3.12**


The adrenal medulla contains large quantities of enkephalin (ENK) peptides. There is little developmental data on ENK peptides in adrenal or extra-adrenal tissue. We measured free MET-ENK, total ENK and CA on extra-adrenal tissue (FAD) and fetal and extra-adrenal tissue (FEAD) in rabbits (age 29 days, n=30) and adult adrenal (Add, n=5). MET-ENK was measured by RIA, Total ENK after digestion with trypsin and carboxypeptidase B, and CA by radioenzymatic assay. ENK results are in ng/g and CA are in ng/ml (Mean ± SEM).

MET-ENK Total ENK MET/TOTAL NE EPI (FAD) 2.1±.3 3.6±.3 4.0±.3 3.4±.3 3.9±.3 (FEAD) 22.3±2.3 32.5±3.3 52.0±3.3 31.0±3.3 (Add) 22.5±2.5 28922.2 0.07±2.0 116.4 484.44

Conclusions: 1) Fetal red blood adrenal and extra-adrenal tissue have large amounts of proenkephalin derived peptides; 2) Post-translational processing (MET/TOTAL ratio) is significantly different in immature rabbits. Speculation: Extra-adrenal tissue is an important source of ENK in fetal and neonatal life.

**3.13**

Expression of the Preproenkephalin A Gene and Related Peptides in Hearts of Spontaneously Hypertensive Rats. M. Durso, M. Ouellette, L. Brasier-Ongaro and J. Lemire. Department of Pharmacology, University of Ottawa, Ottawa, ON K1H 8M5, and Department de Biologie, Université de Montréal, Montréal, QC C33 3J7.

The expression of the preproenkephalin A gene (Enk gene) was investigated in the heart of Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats. The level of Met-Enk was measured by radioimmunoassay and the relative abundance of the Enk mRNA was determined by Northern blot analysis. WKY rats did not display any significant change in their cardiac Enk mRNA content with age but their cardiac Met-Enk levels (free and cyclic peptides) were significantly lower in 16 week old animals (free 1.5 to 0.9 pmol/g; freely; from 5.2 to 3.7 pmol/g; cryptic). In SHR rats, the relative abundance of Enk mRNA in the heart increased with age to levels exceeding those found in WKY animals by factors of 2 to 4 fold at 8 and 16 week old, respectively. The increase in 16 week old SHR was localized in the ventricles. Cardiac free and cryptic Met-Enk in SHR rats did not increase with the age of the animal and were identical or lower (25% decrease in cryptic Met-Enk of 16 week-old SHR) than those found in WKY rats. The striking lack of parallelism between the ventricular levels of Enk mRNA and of free and cryptic Met-Enk suggests that the expression of the Enk gene into Enk-related peptides is suppressed at a translesional level both in control and hypertensive animals. Supported by HSFO.

**3.14**


C-terminal proteinolysis profoundly alters activity in the CNS anesthetic system by converting the opioid receptor agonist β-endorphin (BE) 1-31 to the potent antagonist, β-endorphin 1-27. There have been examinations of effects of this processing in cardiac medulla on central cardioregulation. The cisterna magna was exposed in rats prepared with adrenal vein and femoral artery catheters. BE 1-31, a beta-endorphin tetrapeptide, was infused intracranially. At 30 min postinfusion, baseline mean arterial pressure (MAP=94.7±1.9mmHg) was reduced 24% by BE 1-31, and 32% by BE 1-27, and both peptide altered heart rate (HR=366±15beats/min). The peptides elicited differential compensatory increases in adrenal catecholamine release, that is norepinephrine processing for BE 1-31 (7-fold vs CSF), and with epinephrine for BE 1-27 (4-fold vs CSF). These findings underline the importance of regionally and temporally distinct processing of BE 1-31 on functional specificity. Supporting Grants:USAMRDC 6BIP6813 & NIDA DA0558

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1996
423.15

PRO-OPIOMLANOCORTIN (POMC) mRNA IN THE NUCLEUS TRACTUS SOLITARIUS AND OTHER EXTRAHYPOTHALAMIC BRAIN REGIONS. D.M. Bronstein, M. K.-S. Schafel*, K. A. Trujillo, S. J. Watson and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109

While a variety of techniques have clearly established that the major group of POMC cell bodies in the CNS lies in the arcuate nucleus of the hypothalamus, the presence of extrahypothalamic POMC cells in the CNS is less certain. A small diffuse cluster of POMC cell bodies has been localized in the nucleus tractus solitarius (NTS) area of the caudal medulla, using immunohistochemical methods, but POMC mRNA has never been detected in this region; conversely, while POMC mRNA-like signals have been found in a number of other extrahypothalamic brain sites (e.g., amygdala, cerebral cortex), they give no anatomical evidence of POMC soma in these regions. In the present study, we used biochemical (Northern and RNA protection analysis) and anatomical (in situ hybridization) techniques to determine the distribution and regulation of POMC mRNA in extrahypothalamic brain regions. In addition to the arcuate nucleus, we detected POMC mRNA in the nucleus accumbens, and NTS by in situ protection assays using a riboprobe complementary to the 5' end of exon 3 of POMC mRNA. In order to further characterize POMC mRNA in the amygdala or cortex than is the arcuate message, we found no apparent difference in the size of the POMC message from different brain regions. Using in situ hybridization, POMC mRNA signal was detected in a small number of cells in the NTS following 1-2 months exposure. The number of POMC mRNA copies per cell appears to be lower than the NTS than in the arcuate nucleus. That we observed a relatively strong POMC mRNA-like signal in the caudate and nucleus accumbens with biochemical methods but no signal (to date) using in situ hybridization may indicate a diffuse distribution with low copy number in these tissues. We are currently examining whether extrahypothalamic and arcuate POMC mRNA are identical species and whether they are regulated in similar ways. Supported by NIGA DA02255 and NIMH MH42231 (S.W. & R.A.) and NIH training grants (D.M.B. & M.K.-S.S.).

423.17

PERIPHERAL EFFECTS OF VARIOUS OPIOID AGONISTS ON NEUROGENIC EXTRAVASATION IN SKIN OF THE RAT HIND PAW. A. Barber*, R. Goshchich* and A.P.F. Haase. Pharma Research, E. Merck, Frankfurter Utrasse 250, D-6100 Darmstadt, FRG.

Several reports have indicated that peripheral opiate receptors may mediate some of the analgesic actions of opiates. To address this question, we examined plasma extravasation in rat hind paw skin as a model of neurogenic inflammation. Cut saphenous nerves were stimulated (5 mA, 1 msec, 2 Hz, 10 sec) anterogradely in anaesthetized, Evans Blue-dyed rats. Extravasation was determined by measuring the level of dye in the skin after extraction. Drugs were administered i.v. Morphin and the ε agonist (+)-U50488H produced a dose-dependent inhibition (10-6, 2.4 and 0.68 mg/kg, respectively) of plasma extravasation. The action of 5 mg/kg morphine and (+)-U50488H was completely reversed by 1 mg/kg naloxone, which is indicative of an opiate-mediated effect. (+)-U50488H was ineffective at 1 mg/kg, and produced only 35% inhibition at 5 mg/kg thus demonstrating the stereoselectivity of this effect. 5 mg/kg of the δ/μ agonist DADLE reduced extravasation by 64%, whereas the δ agonist DPPE and the ε agonist (+)-SR 10047 were inactive at this dose. The results presented are therefore consistent with the view that μ and ε, but not selective δ or σ agonists, can inhibit neurogenic inflammation in the skin. Whether this effect is mediated by peripheral opiate receptors on nerve endings of primary afferent fibres, or an indirect systemic action of opiates remains to be determined.

424.1


The bed nucleus of the stria terminals (BNST) plays a critical role in the modulation of the defensive behavior. This modulatory function is also directly influenced by opioid peptides administered to the BNST. Our aim was to examine how BNST neurons respond to opioid peptides and their antagonists. In order to understand the cellular level, the mechanisms of action of these substances. Rats were anesthetized with chloral hydrate (400 mg/kg) and recordings were obtained from these neurons using either single or multibarrel pipettes. Responses to D-Ala2-Met5-enkephalamide (DAME) (10 nm) and naltrexone hydrochloride (50 nm) were examined. Extracellular responses of BNST neurons following microiontophoretic application of DAME showed predominantly excitatory effects, with decreases (50%) firing rates above baseline levels. These responses were blocked by naloxone. More rarely, inhibitory responses could be elicited by DAME and the nucleus accumbens and these showed a potentiation as the duration of the spike discharge. [Supported by NIH grant NS 07941-20 and the UMDNJ Foundation].

424.2

DOPAMINE OVERFLOW IN N. ACCUMBENS FOLLOWING OPIOIDS AND ELECTRICAL BRAIN STIMULATION AS ASSESSED BY MICRODIALYSIS IN FREELY MOVING RATS. Margaret E. Hamilton and Albert A. Spindel. Bethesda, MD. 20892

Previous investigation has demonstrated a failure of morphine to increase extracellular levels of striatal dopamine (DA) in conscious rats. In contrast, other research has indicated that the activity of VTA - n. accumbens DA neurons and concomitant DA release may be increased by opioid agonists. Typically, increased DA transmission in the n. accumbens is associated with enhanced locomotor activity. Acute systemic administration of morphine to naive rats produced only modest effects at the cellular level. Initially, the sedative effect of morphine prevailed. As this effect subsided, however, a slight increase in DA was observed, accompanied by an increase in the number of DA neurons responding to DAME. Pretreatment of rats with the DA uptake inhibitor, nomifensine, attenuated both the sedative phase and the second mild DA increase; however the increases in DA by morphine were proportionally similar to those in rats that did not receive nomifensine. Microinjection of the VTA of the met-enkephalin analogue, DALA, also elicited behavioral activation and moderate DA overflow in the n. accumbens. Similarly, electrical stimulation of the VTA with intermittent trains of biphasic pulses (150 µsec width, 20 Hz at 250 µA) increased both measures in a manner similar to the effects of opioids. It appears, therefore, that a relatively modest elevation of DA in the n. accumbens may be required to enhance behavioral activation. Alternatively, non-DA systems presumably originating in the VTA and perhaps involving endogenous opioids may contribute significantly to behavioral arousal.

Opioid (DA) and opioid peptides were superfused onto ventral tegmental area (VTA) neurons in slices cut horizontally from rat brains. Intracellular recordings were made from 53 cells: 76% were hyperpolarized by DA (1-100 nM) and 19% were hyperpolarized by methionine-enkephalin (ME, 0.1-30 nM). None were hyperpolarized by both DA and ME. Effects of ME were mimicked by D-β-endorphin (0.3-3 nM), but not by DPDPE (3 nM) or U50488H (3 nM), suggesting activation of mu receptors. Electrophysiological properties of DA- and ME-responsive cells resembled primary and secondary neurons, respectively, described in the substantia nigra compacta (J. Neurosci. 9:1233-1241, 1989).

Electrical stimulation of the rostral VTA evoked synaptic potentials (SP's) that were partly blocked by APV (30 nM) and CNQX (10 nM), and partly blocked by picrotoxin (100 nM). ME reduced both the glutamate and GABA components of the SP by 30-40%, but only at high concentrations (300 nM). Spontaneous SP's, evoked by raising K+ to 6.5 or 8.5 mEq/L, were depolarizing with GABA-filanced electrodes, hyperpolarizing with acetate-filanced electrodes, and blocked by bicuculline (30 nM). ME (0.3-3 nM) reduced the frequency of SP's by more than 50% in 2 of 3 neurons. We conclude that opioids act at mu receptors to hyperpolarize GABA interneurons thus reducing inhibitory input on DA-responsive neurons in the VTA.


The nucular locus coeruleus (LC) is densely innervated by libretorsfunctional immunoreactive for opioid peptides. However, the origin(s) of these inputs has not been determined. In the present study, we combined retrograde transport and immunohistochemical techniques to identify the sources of enkephalin (Enk) inputs to the LC. The retrograde tracer, choleratoxin B coupled to colloidal gold particles (CTB-gold), was injected into the electrophysiologically identified LC nucleus. After survival for 5-7 days, the rats received colchicine (100 µg, icv) and were perfused 24 hours later. Results for retrograde labelling with injections restricted to LC confirmed previous reports that major afferents to LC derive from the nucleus paragigantocellularis (PGr) in the rostral ventrolateral medulla, and nucleus raphe magnus (NRh) in the dorsal raphe medulla. Interestingly, the distribution of Enk cells in the PRh was confined to the mediodorsal portion which contains LC afferents. In the ventrolateral medulla the PGr was divided into numerous subeportions, including one on the rostral portion of PGr which contains LC projecting neurons. Examination of Enk and gold labelling in the same tissue sections revealed numerous doubly-labelled cells in both PGr and PRh. These enkephalinergic pathways from the rostral medulla could represent an anatomical substrate underlying opioid effects on LC neurons during stress, opiate abuse and tolerance.1

Support: NIEHS grants NS 24698 and DA 06214 and Fonds de la Recherche en Santé du Québec.

Systemic methionine enkephalin (ME) increases the extracellular norepinephrine concentration in the rostral ventral lateral medulla (RVLM) of the rat. P.A. Mason, J.A. Sterling, S. Testa and H.M. Ruben. Dept. of Anatomy, Cell Biology and Neuroscience1 and Pharmacology, Oral Roberts University, Tulsa, OK 74171.

Our objective was to determine the effects of systemic ME on the extracellular concentration of norepinephrine (NE) in the central site of cardiovascular regulation. Rats were anesthetized and the femoral area and vein of each rat were cannulated. A microdialysis probe was implanted in the RVLM and perfused for 2 hr to obtain baseline data. The rat then received a bolus injection of saline (30 µl IV) followed 40 min later by a bolus injection of ME (300 µg/kg/300 µl IV). At the end of each experiment, glutamate (0.16 mg/10 µl/5 min) was perfused through the probe to verify indirectly the probe's placement in the RVLM. ME decreased SP without a reflex-mediated increase in heart rate. HPLC with EC detection showed that ME significantly increased the NE concentration but did not change the concentrations of DOPAC, HVA and 5-HIAA. Saline had no effect. Glutamate increased the MAP from 87 to 103 mm Hg. These results are consistent with the concept that systemic ME decreases SP via altering the NE concentration in the RVLM.


Noradrenergic neurons of the nucular locus coeruleus (LC) in morphine-dependent rats are strongly activated by opiate-withdrawal (OW). We examined possible mechanisms for such LC activation using extracellular recording of LC unit activity in halothane anesthetized rats.

There was no evidence that hyperexcitability of LC neurons caused their activation during OW, as chronic morphine (i) did not affect the activation of LC cells by ketoprofen pretreatment (1119), and (ii) reduced the excitation of LC cells by sciatic nerve stimulation (by 27%, p<0.05, n=11). Possible involvement of the ventrolateral medulla (VLMM, containing the nucleus paragigantocellularis, the major afferrent to LC) in OW-activation of LC was examined with local injections of the opiate antagonist naloxone (NLX), to induce an excitatory amino acid response (EAA) and then significantly activated LC neurons (2.3 fold, 24/35) in dependent rats. This effect is specific (i) to dependent rats (23/27 in naive rats), (ii) to opiate receptors as the EAA failed to induce neurons in the inactive enantiomer (NLX=0), and (iii) to VLMM as injecting NLX to active site in the same animal (n=5) or in LC (n=5) were ineffective. Smaller doses of NLX injected into VLMM produced only weak excitations of LC (10-9 to 10-4 M) and the activation of LC cells by local or systemic NLX was blocked by kynurenate (an antagonist of excitatory amino acids, EAAs; 10-2 M) directly infused into LC (n=3). These results indicate that VLMM may be the primary site whereby OW activates LC neurons and that this activation is produced by an EAA input to LC. Supported by NIDA grants NS 24698 and DA 06214 and FRSC.

Regulation of opioid peptide and receptor systems following exogenous opioid administration in neonatal rat brain. A. Tempel. Dept. of Psychiatry, Laboratory of Neuropsycharmacology, Hillside Hospital/LJMC, P.O. Box 36, Glen Oaks, New York 11004.

Opioid analogics are well known to produce tolerance and dependence in vivo and desensitization in vitro. The nature and consequence of these phenomena are not clear. We have found that chronic pre- and postnatal morphine treatment of rat pups produces a significant decrease in brain α opioid receptor density with no change in receptor affinity. This decrease is accompanied by tolerance to the actions of morphine. In order to determine changes in specific brain regions, autoradiography was performed on brain sections from control and chronically morphine treated neonatal rats. The effects of postnatal morphine treatment induced a 30% loss of α opioid receptors in the patches of the striatum with no significant loss in the matrix area. This loss of α receptors was no longer observed with prolonged morphine treatment. These data provide evidence for a unique plasticity of the immature opiate receptor system. In order to determine if the morphine-induced changes alterations in opioid peptide synthesis, preproenkephalin (PPE) mRNA levels were examined in brains of control and chronic morphine treated animals. Chronic morphine treatment produced a significant decrease in PPE mRNA relative to salience treated animals in various brain regions. Data from these studies provide insight into interactions between endogenous and exogenous opioids in opioid tolerance and dependence in the developing central nervous system. (Supported by NIDA grant DA-05440).
424.9 MU TOLERANCE IN ONTOGENY: ANTIOPIOCEPTIVE STUDIES. R.W. Windh and C. Kuhn. Dep't of Pharmacology, Duke University Medical Center, Durham, NC 27710.

The opioid receptor antagonist is an important determinant of the physiologic outcome following developmental opiate exposure. To examine this question, the present study evaluated tolerance at the mu receptor in developing mice using an antioncceptive model. Animals were treated for 5 days with the agonist morphine (3mg/kg-25mg/kg s.c., twice daily) on days 4-8 or 22-26 and challenging rate. Sufentanil was then administered to opiate-naive 10 and 28 day old rats caused a dose-dependent and naloxone reversible increase in latency to paw removal from a hot plate. Chronic treatment had markedly diminished effects at the two ages studied: while the 28 day olds showed no antionceptive response to any dose of sufentanil, the dose-response curve in 10 day olds was unchanged. These results show that mu receptors involved in antioncceptone show a relative inability to adapt to chronic perturbation early in development. This resistance to tolerance contrasts with our previous demonstration of tolerance in corticosterone secretion, suggesting there may be a spectrum of adaptability at various sites. However, both these findings are consistent with a literature indicating less robust tolerance in developing animals, and they suggest that opiates might exert more profound effects on developing animals than on adults after chronic administration. Supported by DA 02739.


This study further examined our previously identified role of coerulospinal noradrenergic pathway in fentanyl-induced muscle rigidity, based on a combined histochimical, immunocytochemical and pharmacologic evaluation, unilateral, site-specific microinjection of fentanyl (2.5µg/50 nl) into the locus coeruleus (LC) of Sprague-Dawley rats anesthetized with ketamine. Sufentanil was antagonized by a pretreatment with the specific µ1-adrenoceptor blocker, prazosin (250 µg/kg, i.v.). The above results were significantly eliminated in animals pretreated with the selective noradrenergic neurotoxin, DSP4 (50 mg/kg, i.p.). There was also an appreciable reduc-


Changes in renal function were examined during left renal artery (Ia) Infusion of the selective µ-opioid agonist, dermorphin, in anesthetized Sprague-Dawley rats with intact (n=6) and bilaterally denervated kidneys (n=6, n=7). Urine was collected from left and right (control) kidneys via ureteral cannulae during 20 min. Ia infusion periods of isotonic saline (0.9% NaCl, 0.05 n/ml/min/g) and isotonc saline filtration recovery measurements for urine flow rate (V) and urinary sodium excretion (Un-V) and renal plasma flow and plasma flow were similar between and during left dermorphin. O(20:2) vs. 0.9% NaCl, 0.05 n/ml/min/g and 3.010-4 vs 2.610-3 m/min/g, respectively. These results suggest that µ-opioid agonists participate in the renal tubular system and water reabsorption via an intrarenal action to facilitate nontactile activation of norepinephrine.

424.12 EFFECTS OF OPIOID AGONISTS AND ANTAGONISTS ON GABA-


Occupancy of the sigma receptor/binding site has been suggested to modulate the ascending dopamine (DA) systems on the basis of the localization of sigma receptors adjacent to nigrostriatal DA cell bodies (Graybiel et al., 1988) and the behavioral activity of positive and negative neuroleptics (Taylor et al., 1988). We utilized microstructural analysis of lick patterns to examine the actions of the selective sigma agonist (+)-SKF 10,047, 5 mg/kg, BMY 14082, 4 mg/kg, and compared them with the actions of naltrexone on sucrose reinforcement. Six male 5-D rats with chronic gastric fistulas were adapted to 30-min tests shan feeding a 20% sucrose solution after an 18h food deprivation and injection of naltrexone. We observed that naltrexone was not the case for other selective sigma drug on volume shan fed. In contrast, haloperidol produced a highly significant reduction in shan intake (33.7 ± 2.7 ml to 15.0 ± 2.7 ml, p<.001). However, BMY 14082, but not SKF 10,047 or haloperidol, produced a significant decrease in interlick intervals from 0.9260 (p<.005) as well as in the number of licks per ml consumed (p<.05). No other microstructural changes were produced by the selective sigma agonist, the effects of haloperidol on other microstructural measures confirmed our previous studies (Schneider et al., 1989). These results suggest that sigma receptors do modulate dopaminergic systems which are critical to the reinforcing potency of sucrose. Supported by NIMH RSA MH400149 (GPS), NIH R29 NS24781 (LHS), and NIDA RO1 DA 05782 (BRM).

424.14 SIGMA LIGAND PENTAZOCINE (PENT) INCREASES EXCITABILITY IN HIPPOCAMPAL NEURONS. A.E. Cole, J.J. Arvanpur, C.U. Eccles and R.S. Fisher. Dept. of Neuro, Johns Hopkins Hospital, Baltimore, MD 21205 and Dept. of Pharm. and Toxicol., Univ. of Maryland School of Pharmacy, Baltimore, MD 21201.

The sigma receptor is a novel site implicated in psychoses and epilepsy. The sigma receptor ligand PENT has been shown to increase field potential amplitude at low concentrations (1-10 uM) in the in vitro hippocampal slice (Elgersma et al., 1989). The present study examined the effect of PENT on intracellularly recorded synaptic responses in hippocampal pyramidal neurons of region CA1. In the presence of 10 uM PENT the amplitude of the IPSPs evoked by orthodromic stimulation of the Schaffer collateral pathway increased by 150% of control values. No significant effects were observed on the resting membrane potential or input resistance. At the same time, the IPSPs was preserved in all cells examined. In additional neurons where KCl-filled electrodes were perfused with PENT there was an increase the frequency and amplitude of spontaneous IPSP's. In all experiments PENT was applied by bath perfusion; APV and naloxone were added to block potential interactions with NMDA and opiate receptors, respectively. These results demonstrate that PENT interacts with synapses excitability of hippocampal neurons. The data suggest a physiological action of sigma receptors on synaptic function in rat hippocampus.
**425.1**

**THYROTROPIN-RELEASING HORMONE (TRH) mRNA IS INCREASED IN SPECIFIC LIMBIC SUBREGIONS FOLLOWING ELECTROCONVULSIVE SEIZURES (ECS) AS DETERMINED BY IN SITU HYBRIDIZATION HISTOCHEMISTRY.** J.A. Fussman, J.R. Ardeleto, A. Sattin and N.J. Feeney. Deps. of Anatomy, Psychiatry and Psychology in Medical Neurobiology, Indiana University & VA Medical Center, Indianapolis, IN 46222. Previous studies have shown that induction of limbic seizures by kainic acid (KA) produced large increases in the concentration of TRH in limbic regions of the rat CNS. (Regul. Physiol. & Biochem. 28:183-93, 1990). These increases are differentially attenuated by dizapam pretreatment (McCormick et al., Neurosci. Abs., 12:1399, 1990). We investigated whether the NMDA antagonist MK-801 would prevent KA induced elevations in TRH content.

Male Sprague-Dawley rats (180-200g) were sacrificed 5 days later and regional TRH content determined by radioimmunoassay. MK-801 prevented the KA induced increase in TRH in the corpus striatum and posterior cortex. KA-induced elevations in dorsal hippocampus were attenuated by MK-801, while elevations in ventral hippocampus were not affected. In the amygdala/parafimb cortex, KA induced elevations in TRH were enhanced by MK-801. These results indicate that there is a differential effect of MK-801 on the responsiveness of TRH systems to limbic seizure activity.

Supported by NIMH 55641 and VA.

**425.2**

**NUCLEATION OF LIMBIC SEIZURE INDUCED ELEVATIONS IN TRH CONTENT BY MK-801.** N.A. Erdreider and A. Winkmuller. Dept. of Psychiatry, Univ. of PA, Philadelphia, PA 19104. Using in situ hybridization histochemistry, we have shown that induction of limbic seizures by kainic acid (KA) produced large increases in the concentration of TRH in limbic regions of the rat CNS. (Regul. Physiol. & Biochem. 28:183-93, 1990). These increases are differentially attenuated by dizapam pretreatment (McCormick et al., Neurosci. Abs., 12:1399, 1990). We investigated whether the NMDA antagonist MK-801 would prevent KA induced elevations in TRH content.

Male Sprague-Dawley rats (180-200g) were sacrificed 5 days later and regional TRH content determined by radioimmunoassay. MK-801 prevented the KA induced increase in TRH in the corpus striatum and posterior cortex. KA-induced elevations in dorsal hippocampus were attenuated by MK-801, while elevations in ventral hippocampus were not affected. In the amygdala/parafimb cortex, KA induced elevations in TRH were enhanced by MK-801. These results indicate that there is a differential effect of MK-801 on the responsiveness of TRH systems to limbic seizure activity.

Supported by NIMH 55641 and VA.

**425.3**

**Vasoactive Intestinal Peptide (VIP) in the Normal and Decentralized Superior Cervical Ganglion (SCG).** H. Hijiyama-Saeki, M.C. Beisert*, C. Baldwin and R.E. Zigmond. Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44106 and Department of Pharmacology, St. Louis University, St. Louis, MO 63104

The rat SCG has been shown by immunohistochemical techniques to contain VIP-like immunoreactive (VIP-IR) neural processes and a small number of immunoactive cells (J. Comp. Neurol. 220:521, 1988). Double-labeling studies have demonstrated that VIP-IR is also present in cell bodies of preganglionic sympathetic neurons that project to the gut (J. Comp. Neurol. 325:55, 1990). Finally, ligations of the preganglionic sympathetic trunk lead to the accumulation of VIP-IR both on the spinal cord side and on the ganglion side of the ligature. These data indicate that some, but not all, of the VIP-IR in the SCG is present in preganglionic afferent fibers. In the present study, VIP-IR was determined by RIA of acetic acid extracts of SCG. The phosphorylated assay was assayed for VIP-IR using a VIP anti- serum from Professor Shigi (Kyoto, Japan). The assay yielded a dilution curve that was parallel with authentic VIP. The content of VIP-IR in SCG from normal rats was 11.6 pg/g (12.5 pg/g extracted protein; N=26 ganglia). When the SCG extract was run on HPLC, VIP-IR was detected only in those fractions corresponding to the retention time of authentic VIP. VIP-IR was also measured in the SCG 48h after cutting the cervical sympathetic trunk. The level of VIP-IR was increased by 2 fold in decentralized ganglia compared to ganglia from both sham-operated and sham-operated animals. Examination of sections of the SCG six weeks after decentralization revealed an increase in the number of immunostained neural cells and no apparent change in the number of immunostained neuronal cell bodies. These data suggest that there is an increase in VIP in VIP positive cells to the SCG or, possibly, in postganglionic neural processes following section of the preganglionic afferent trunk (NS10651, NS10687 and MH01062).

**425.4**

**The Probable Identity of a Neuropeptide Metabolizing Enzyme with the Lysosomal “Protective Protein”.** R.L. Jackman, F. Tanu, T. Tamii, G. Seuring-Harburt, R.A. Zigmond, B.G. Fyffe, Lab. of Peptide Research, Dept. of Pharmacology, Univ. of Ill., Coll. of Med., Chicago, IL 60612

Most of the actions of neuropeptides depend on intact C-terminal amide (Nε-Dep). We extracted an enzyme from human platelet granules that deamidates substance P and other neuropeptides. The enzyme, released from human platelets by thrombin, was purified to homogeneity. The purified deamidase has a molecular weight of 52kDa and consists of 2 chains, a 33 kDa and a 21 kDa chain. The enzyme is a serine proteinase; "P28kD" labels the active site serine on the heavy chain. The purified enzyme has esterase, peptidase and peptidase activities. With biologically active peptide, the enzyme acts both as a deamidase (substance P, neuropeptide A and leedosin) and as a carboxypeptidase (bradykinin, angiotensin I) at neutralpH, although the carboxypeptidase action is faster at pH5. After sequencing the first twenty-five amino acids of both chains, an identical sequence was found in the corresponding two chains of lysosomal "protective protein." A defect in this protein is the cause of galactosialidosis, a severe genetic disorder with progressive neurological deterioration and mental retardation. (These studies were supported by NIH: HL35082, HL36473, HL36081, DK41341.)

**425.5**

**Calcium-Binding Proteins in Aplysia Neurons.** A. Herrman (1), T.S. Pauly (2) and C.W. Helmsen (3). (1) Univ. of Salzburg, Dept. of Zoology, A-8020 Salzburg, Austria; (2) Univ. of Zurich-Lachel, Inst. of Pharm., Bülach, CH-8032 Zürich; (3) Univ. of Zurich, Dept. of Pediatrics, CH-8032 Zürich, Switzerland.

Protein pattern of single, identified neurons (burrset, beating and sminle within the neuromuscular system of the nudibranch Aplysia californica were studied by 2D-Page. Major differences between neurons were found in the range of low molecular weight and low isoelectric point, where Ca-binding protein were more clearly localized. Ca2+ ion experiments revealed a prominent protein group for binding 15 cells which migrated at an identified position to carry 2 calcium (Ca2+) neurons, whereas a protein spot characteristic to all 15 cells which migrated at an identified position to carry 2 calcium (Ca2+) neurons. Western blot analysis did not show immunoreactivity in the molecular range where PV-like proteins are found. Immunoreactivity was observed, however, with a different protein at Mr 40,000, pl 4.8. The same protein also coeluted with an antiserum directed against rat calbindin D-28k and in addition bound high amounts of Ca2+ as revealed by transblot/Coomassie technique. The results suggest that this protein is a novel Ca-binding protein sharing common antigenic determinants for both, PV and calbindin D-28k.

In heat treated extracts of ganglia we further identified a group of proteins with Mr 13000-20000, pl 4.6, immunoreactive, to antibodies against Ca-binding protein II of the Ca channel. These proteins which share a high degree of similarities to 92% therefore appear to represent isomeric forms of these proteins in neurons.

Supported by NIMH fellowship ZF 5696 and the Swiss National Science foundation (31-94/98).
425.7


We recently observed that fluoramidine (FLU) protects serotonergic systems from the neurotoxic action of 3,4-methylenedioxyamphetamine (MDMA). The purpose of this study was to determine if administration of FLU in a manner that also induced amphetamine (MET) or MDMA-induced changes in extrapyramidal neurotransmitter systems. Male Sprague-Dawley rats (180-250 g) were injected with 4 doses of MET (15 mg/kg s.c.), MDMA (10 mg/kg s.c.) or saline at 6-hour intervals with or without prior FLU (30 mg/kg, i.p.) administration. The animals were killed 18 h after the last drug administration. FLU administration alone resulted in a 36% increase in striatal neurotransmitter-like immunoreactivity (NTLI) while having no effect on the nigral levels. METH and MDMA increased striatal NTLI to 187% and 148% of control, respectively; nigral concentrations were increased to 579% and 441%, respectively. FLU treatment potentiated the METH and MDMA responses in the striatum while failing to alter the nigral changes. The responses are similar to the effects of a dopamine D2 receptor antagonist on the METH-induced changes, thus suggesting that FLU affects D2 receptor transmission. This conclusion is supported by the observation that FLU increases dopamine metabolism 3 h after a single administration. These results demonstrate that serotonergic and peptidergic changes resulting from MDMA treatment are differentially mediated. (Supported by USPHS grants DA 00869 and DA 04222)

425.9

SECRETION OF CARBOXYPEPTIDASE H FROM AIT-20 PITUITARY TUMOR CELLS. D. Parkinson, Dept. Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110.

The biosynthesis of peptide transmitters requires the proteolytic processing of precursor by several enzymes. In order for these enzymes to function, they must be packaged along with the precursor into the secretory vesicles where the appropriate milieu is maintained. Carboxypeptidase H (CPH) removes C-terminal basic amino acids from partially-processed precursor. To gain insights into the mechanism of sorting of proteins into the secretory vesicle, the secretion of CPH has been investigated in AIT-20 mouse pituitary tumor cells.

AIT-20D-16 cells were grown to near-confluence in DME supplemented with 10% Nu-Serum. Media was collected and analyzed for CPH content by enzyme assay or western blotting. AIT-20 cells secrete enzymatically-active CPH constitutively. The accumulation of CPH activity was slow over a 10 hour period. This activity was stimulated 3-5 fold after cell death. The increased secretion of this enzyme is probably by CPH-selective inhibitors. CPH secretion was stimulated 3.6 fold by serotonin. There was a direct correlation between CPH activity and the opening of a 140 kD band on western blotting with an antibody directed to the C-terminal sequence. These results show that CPH is not processed at its C-terminus. To test the importance of acidic compartments in the secretion of CPH, cells were treated with ammonium chloride. This treatment produced small increases in secreted CPH activity. This result suggests that constitutively-secreted CPH is active, regardless of whether it has passed through any acidic compartments.

425.11


Apafia egg-laying hormone (ELH) was chemically modified using an N-hydroxysuccinimide (NHS)-Long Chain (LC)-Biotin reagent. Sites of modification were analyzed by FAB-MS, HPLC, amino acid composition analysis and protein sequence analysis. Several species of biotinylated ELH were produced, owing to the variable reactivity of the N-terminal α-amino group and the two ε-amino groups of Lys and Lys*. The ELH-Lys*, Biotin species was found to be biologically active and equipotent to native ELH in an egg-laying bioassay and in an in vitro neuro assay using cell line R15 and identified locations in the abdominal ganglion. Analysis of the reactivity of the primary amines indicated that Lys* was significantly more reactive than Lys or the N-terminal. These results are consistent with the first report of a chemically labeled, biologically active ELH molecule. With the development of biotinylated ELH, it is possible to pursue studies in receptor binding and target tissue localization. Second, lower reactivity of the N-terminal amino group suggests that it may not be as accessible as the ε-amino group of Lys*. This reduced access could be attributed to a mechanism that renders the peptide less susceptible to aminopeptidases and, therefore, could contribute to an increased lifetime for ELH in the animal.

425.10

METABOLISM OF EXTRACELLULAR N-ACETYLSPARTYL-"H"GLUTAMATE AND ITS PRODUCT "H"GLUTAMATE IN INTACT CELLS OF MURINE WHOLE BRAIN PRIMARY CULTURE. M. Cussady and J. M. Walcarius, Dept. of Pharmacology, Georgetown University, Washington DC 20057

N-Acetylaspartylglutamate (NAAG) may function in synaptic communication in the mammalian CNS. It is present in synaptic vesicles and released on stimulation in several systems, yet the extracellular fate and effect of this peptide remain unclear. The goal of this study is to define the extracellular metabolism of NAAG and the fate of both NAAG and glutamate in intact cultures of murine brain. NAAG and glutamate cultures of murine brain were incubated with N-acetylaspartyl-"H"glutamate in the presence and absence of inhibitors of NAAG peptidase activity. Data suggest that NAAG is rapidly degraded by a cell-surface enzyme to N-acetylaspartate and "H"glutamate. Initial results indicate that in a whole cell system, "H"glutamate, and to a much lesser extent, intact "H"NAAG, are taken up by cells. This suggests removal of NAAG from the medium by both degradation and limited direct uptake. The role of NAAG in communication may be two-fold: as a rapidly acting neuromodulator or as a chronic source of glutamate in the synaptic cleft, serving to modulate neuronal excitability.

425.12


Recently we observed marked increases in brain levels of neuropeptide Y (NPY) and NPY-RNA after kainic acid (KA) induced seizures in the rat. By measuring incorporation of locally injected "H-tyrosine, we now found a markedly enhanced biosynthesis of NPY and NPY-RNA in KA treated animals. This increased rate of NPY in the cortex 2 to 30 days after KA. We furthermore investigated changes of NPY immunoreactivity and gene expression after KA kindling in both animal models pronounced increases of NPY immunoreactivity were observed in the terminal field of mossy fibers. In KA kindling, NPY mRNA was strongly expressed in the molecular layer of the dentate gyrus after 30 to 60 days, suggesting neuronal sprouting. Unilateral injection of colchicines abolished NPY mRNA expression in the mossy fibers. Using "in situ" hybridization, in both animal models markedly enhanced expression of preproNPY mRNA was observed in the granule cell layer. It is suggested that sustained expression of the neuropeumonial peptide NPY, in addition to the observed plastic changes, may contribute to altered excitability of hippocampal mossy fibers in epilepsy. Somatostatin immunoreactivity and gene expression were not changed in mossy fibers or granule cells, respectively.
PEPTIDES: BIOSYNTHESIS AND METABOLISM II

425.13


Recently we observed increased mRNA levels of neurokinin B in the frontal cortex and hippocampus after kainic acid induced seizures and after pentyleneetetrazol kindling in the rat. Using immunohistochemistry and "in situ" hybridization we now investigated changes in neurokinin B immunoreactivity and in mRNA encoding for neurokinin A and B, respectively. In both animal models we observed pronounced increases in neurokinin B immunoreactivity in granule cells, in the terminal field of mossy fibers and in pyramidal cells of the CA1 sector. According to this a also markedly enhanced expression of prepro-neurokinin B mRNA was observed in the granular layer as well as in pyramidal cells of the CA1 sector. Enhanced neurokinin B immunoreactivity and prepro-neurokinin B mRNA were also found in the frontal and granular cortices (mainly in layer 2). Prepro-neurokinin A mRNA was present in high concentrations in neurons of the habenula, but only in minute amounts in the hippocampus. It was unchanged in epileptic rats. Our experiments suggest enhanced induction of neurokinin B but not of neurokinin A gene expression in both animal models of temporal lobe epilepsy.

425.15

ISOLATION AND CHARACTERIZATION OF DROSOPHILA PEPTIDES CONTAINING ArgPheNH2 C-TERMINUS, R. Nichols and M. Conkright*. Departments of Biological Chemistry and Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

 Naturally-occurring Drosophila peptides containing -ArgPheNH2 C-terminal sequences have been identified and characterized. Purification of peptides from crude homogenates were monitored utilizing a radiolmmunoassay specific for -ArgPheNH2. Various chromatographic methods based on size and charge were used to purify the peptides. "Bombesin" (DSK) and three peptides from pro-FMRF were isolated. A novel peptide was also identified. The structures of the purified peptides were determined by automated Edman degradation and fast-atom bombardment mass spectrometry (FABMS). A number of other immunoreactive peaks have been identified and their structures are being characterized. It is anticipated that many of the peptides will correspond to previously unreported forms of DSK, FMRFamide or related peptides.

SEROTONIN III

426.1


Monkeys administered (±)-4,5-methylenedioxyamphetamine (MDMA) show large (75-90%) depletion of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) two weeks later (JAMA: 280: 31-15, 1998). Results on weeks, partial recovery, find that 3-5HT and 5-HIAA are depleted by only 30-47% (Neurosci. Abst., 14: 509, 1988). The purpose of the present study was to determine if serotonergic recovery in the MDMA-treated primates continues over time and, if does, to ascertain whether it is complete by 18 months.

Six squirrel monkeys (Saimiri sciureus) were administered MDMA hydrochloride at a dose of 7 mg/kg (twice 800 mg/kg and twice 1700 hrs) for 4 days. Three of these animals were killed 7 months later; the remaining 3 were sacrificed 18 months later. Regional brain 5-HT and 5-HIAA determinations were performed using HPLC-EC methods, with the two treatment groups being assayed in parallel. Relative to untreated controls (6), MDMA-treated monkeys at both survival periods (7 and 18 months) showed substantial reductions in both 5-HT and 5-HIAA (32-70% depletion, depending on brain region in both). Notably, there was no evidence of recovery between 7 and 18 months.

These results suggest that 5-HT neural damage induced by MDMA in the primate is long-lasting, possibly permanent. We suggest that the partial recovery of 5-HT which takes place in the monkey occurs within the first few weeks after MDMA exposure. Given that the regenerative capacity of central 5-HT neurons in the primate is apt to be limited, these findings could have important public health implications since use of MDMA continues. [Supported by DA15070 and in part by MAPS]

426.2

EVALUATION OF THE NEUROTOXIC POTENTIAL OF 2-HYDROXY-4,5-METHYLENEDIOXYMETHAMPHETAMINE (2-HOMDMA), A REPORTED MEТАТАBOLITE OF MDMA. Z. Zhao, G. Ricaure and N. Castagnoli. Jr. Dept. of Chemistry, Virginia Tech, Blacksburg, VA 24061 and *Dept. of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21224.

The serotoninergic monkey MDMA [2-methylamino-1-(3,4-methylenedioxyphenyl)propane] is reported (Lim and Pelto. Soc. Tot. Abst., 13: 333, Feb. 1990) to be metabolized to 2-HOMDMA which is a possible precursor to the corresponding hydroxyamphetamine analog, a potentially neurotoxic agent. The systemic and full characterization of this MDMA metabolite as a racemate have been accomplished via gas-liquid chromatography, mass spectrometry of hydroxyamphetamine derived via nitrostyrene chemistry, and 2-amino-2-methyl-1-(3,4-methylenedioxyphenyl)ethanol, which in turn was protected against the primary amine with LIAH3. Reaction of this amine with ethyl formate followed by LIAH3 reduction of the resulting formamide and hydroxyacetamide to remove the benzyl protecting group and to the desired product which was obtained as a stable, crystalline hydrochloride salt. Systematic administration of 2-HOMDMA to male Sprague-Dawley rats for a dose 4 times greater than the amount required to cause a 60-70% depletion of regional brain serotonin markers one week later was without effect. Similar results were obtained following intravenous administration. Further, they suggest that the partial recovery of 5-HT which takes place in the monkey occurs within the first few weeks after MDMA exposure. Given that the regenerative capacity of central 5-HT neurons in the primate is apt to be limited, these findings could have important public health implications since use of MDMA continues. [Supported by DA15070 and in part by MAPS]
463.3

EFFECTS OF MDMA ON HIPPOCAMPAL AND BRAIN STEM 5-MT ACTIVITY


Monoamine oxidases are mitochondrial-bound enzymes which break down monamines such as serotonin (5-HT) in the nerve cell body. Now clorgylline and yohimbine are drugs which inhibit MAO and have been used as mood-elevating drugs in the treatment of depression. 3,4-methylenedioxyamphetamine (MDMA) is a designer drug which produces euphoria, and has been shown to affect 5-HT both by potentiating release and blocking uptake. The effects of MDMA on MAO activity in 5-HT terminals were studied to determine whether MDMA could also potentiate the above-mentioned effects by acting as an MAO inhibitor. The hippocampus and brain stem of these animals. There were no differences in sensitivity to the drugs between adult rats and young rats were removed and placed in 10x v/v 0.01M PB. Following homogenization and centrifugation, the remaining tissue was resuspended in 0.01M PB and incubated with 3H-5-HT, and MAO in a range of concentrations (10^-7 - 10^-9) at 37°C. MAO activity was measured by the scintillation counting of E-5H-CT. An IC50 of 6x10^-6 was found on MAO activity in both the hippocampus and brain stem of these animals. These results are consistent with the hypothesis that 5-HT, antagonists block MAO-induced neurotoxicity by interfering with MAO-stimulated DA synthesis and that sustained DA release itself is required for MAO-induced neurotoxicity.

464.6

DIRECT MORPHOLOGIC EVIDENCE FOR DEGENERATION OF SEROTONERGIC AXONS FOLLOWING ADMINISTRATION OF AMPHETAMINE DERIVATIVES: di-p-CHLOROAMPHETAMINE, 4-METHYLAMPHETAMINE, AND d- and L-PHENYLLEREFERINE.

Kates J. Art, Thaya M. Teague* and Mark E. Mohler. The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The loss of markers for 5-HT innervation of forebrain caused by psychotropic amphetamine derivatives has suggested that these compounds are neurotoxic. To obtain direct evidence for axon degeneration, we examined the morphologic effects of these drugs at short post-treatment intervals. Following treatment with di-p-CHLOROAMPHETAMINE, 10 mg/kg s.c. for 21 days, significant axonal loss (>50%) was observed in the substantia nigra. The loss of markers for 5-HT innervation of forebrain caused by psychotropic amphetamine derivatives has suggested that these compounds are neurotoxic. To obtain direct evidence for axon degeneration, we examined the morphologic effects of these drugs at short post-treatment intervals. Following treatment with di-p-CHLOROAMPHETAMINE, 10 mg/kg s.c. for 21 days, significant axonal loss (>50%) was observed in the substantia nigra.

465.7

SPECIFIC REGIONAL PATTERNS OF SEROTONINERGIC INNERVATION IN VENTRAL AREAS OF FOREBRAIN IN THE RAT: SELECTIVE DENERVATION AND REINNERRATION AFTER ADMINISTRATION OF P-CHLOROAMPHETAMINE (PCA).

Mark E. Mohler and Karen J. Ast. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The 5-HT innervation of the neocortex exhibits regional specificity in terms of denervation and innervation of aminergic systems. The study examines the 5-HT innervation, PCA-induced denervation, and innervation changes in different brain regions. The 5-HT innervation of the neocortex, and the hippocampus, exhibits regional specificity in terms of denervation and innervation of aminergic systems. The study examines the 5-HT innervation, PCA-induced denervation, and innervation changes in different brain regions.

466.8

P-CHLOROAMPHETAMINE-INDUCED NEUROTOXICITY IN RAT BRAIN DEPENDS ON THE PRESENCE OF ENDogenous SEROTONIN.

U.V. Berger, R. Grasman and M.E. Mohler. Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Systemic administration of p-chloroamphetamine (PCA) causes acute release of serotonin (5-HT) and produces long-lasting degeneration of 5-HT axons in brain tissue. However, direct intracerebral injection of PCA does not produce neurotoxic effects on 5-HT axons, suggesting that a neurotoxic effect is generated in the periphery following systemic administration. This study was designed to test the hypothesis that endogenous serotonin (5-HT) plays a role in mediating the neurotoxic effects of PCA in the brain. PCA was administrated to rats that had been previously depleted of 5-HT by different regimens of p-chlorophenylphthalamine and/or reserpine. Two weeks later, the serotonin innervation of the forebrain was evaluated using immunocytochemical and biochemical methods. The results show that acute depletion of 5-HT using PCA produces substantial protection against the PCA-induced degeneration of 5-HT axons. While drug interactions possibly may have a protecive effect, it is more likely that the neurotoxicity of PCA is dependent on the release of endogenous 5-HT. The results show further that the protective effect is most profound following treatment regimens that are sufficient to deplete peripheral as well as central stores of 5-HT. We interpret these findings as evidence that peripheral stores of 5-HT, i.e., in platelets and enterochromaffin-cells, are necessary for the expression of PCA-induced toxicity. Based on these results, we propose that neurotoxicity induced by p-chloroamphetamine may be caused by a toxic metabolite of 5-HT. This mechanism may also account for the neurotoxicity seen with other psychotropic amphetamines such as 3,4-methylenedioxyamphetamine. [Support: USPHS Grant DA-04431 & PMA]
426.11

Acute intracerebroventricular administration of fenfluramine causes increases in extracellular serotonin (5HT) in cortex, hypothalamus and n.accumbens in anesthetized rats (LaFerriere and Wurtman, 1989). DL-fenfluramine increases cortical 5HT in freely moving rats 2 hours after diazepam treatment (Carboni and Di Chiarla, 1989). In the present experiment, the effect of DL-fenfluramine on extracellular serotonin in the hippocampus was studied in freely moving rats. Rats were permanently implanted with BAS 4 mm dialysis probes (N=5). Fifteen hours later (Day 1) the rats were perfused with Ringers solution (0.20, 10 and after probe implantation. The probe were not removed from the brains during this 10 day period, and the rats were perfused only on the 3 test days. Basal 5HT values were 1.1, 1.1 and 1.1 ug/min on days 1, 5, and 10, respectively; 60 min post-injection, the 5HT values were 11.2, 8.7, and 4.1 pg/min on days 5, 1, and 10. Because chronic fenfluramine is known to deplete SHT tissue levels (Klaven and Seiden, 1989) the increased serotonin response in this study may be due to a mild toxic effect of the 3. 4-methoxyjugans. It is more likely, however, that the probe has become less viable over time. We are currently investigating these possible interpretations. This work was supported by MH-11191 and RSA-10562 (L. Seiden).

426.12
INCREASE IN TRYPHTOPHAN HYDROXYLASE CONCENTRATION IN ADULT RAT NEOSTRIATUM AFTER NEONATAL DOPAMINE DENERVATION WITH 6-HYDROXYDOPAMINE. D. Weissmann*, C. Roussos*, J.F. Pajot, D. KLS, and J.L. Lyons, Université Claude Bernard, Lyon, FRANCE, and CRNS (Département de physiologie), Université de Montréal, Montréal, CANADA H3C 3J7.

Using a recently developed radio-immunoassay technique for the visualization and measurement of enzymatic protein transferred from unfixed brain sections onto nitro-cellulose (Weissmann, D. et al., J. Neurochem. 53:793, 1989), we have examined the human brain hydroxylase (TPOH) content in adult rat neostriatum, globus pallidus and raphe nuclei, after bilateral l.c.v. administration of 6-OHDA to 3-day-old rats pretreated with desipramine or paroxetine. This treatment is an almost complete dopamine denervation of the forebrain and a prominent serotonin (5-HT) hyperserotonine in the rostral half of neostriatum (Snyder, A.M. et al., J. Comp. Neurol. 245:274, 1986). Three to four months after the treatment, a significant increase in tissue TPOH concentration (+26-36%) could be measured in the rostral but not the caudal part of neostriatum. However, there were no changes in TP0H tissue concentration in the globus pallidus nor in the nuclei raphe dorsalis, medianus or pontis. These data strongly suggest that the augmented TPOHontrolation in the 5-HT-hyperinnervated neostriatum was not associated with an increased concentration of the enzyme in the nerve cell bodies of origin. If the turnover rate of TP0H is not reduced in the neostriatum, a greater amount of the protein must be produced and delivered to an enlarged axonal tree, thus maintaining the steady state concentration of the enzyme within the 5-HT nerve cell bodies.

426.13
CORRELATIVE SCANNING AND TRANSMISSION ELECTRON MICROSCOPY OF THE SUPRAEPENDYMYAL NEURONAL COMPLEX IN THE HAMSTER THIRD VENTRICLE FOLLOWING RADIOFREQUENCY LESIONS OF THE MOBRAIN RAPHE NUCLEI. B.D. Felesler*, J.C. Hazlett and J.A. Mitchell, Departments of Anatomy & Cell Biology and Neurosurgery, Wayne State University School of Medicine, Detroit, MI 48201.

Scanning electron microscopy (SEM) of the hamster (Mesocricetus auratus) third ventricle following radiofrequency lesions of the midbrain raphe nuclei (RM) reveals degeneration of the suprapendymal neuronal complex (SENC) within 2-4 days (Fessler & Mitchell, Soc. Neurosci. 15: 419). The current study utilizes correlative transmission electron microscopy (TEM) to determine specifically which elements of the SENC undergo degeneration following lesions of the RM. Hammond third ventricles previously processed for SEM were prepared for examination by TEM. TEM revealed extensive loss of intrinsic ultrastructural elements, including synapses, within the denervated SENC. Complete lesions of the midbrain RM resulted in total loss of the SENC within 2-4 days. Control animals lesioned 1-2 mm caudal to the midbrain RM did not exhibit evidence of SENC degeneration. The results of correlative SEM/TEM following raphe lesions confirm previous SEM documentation of degeneration and suggest that the hamster SENC is dependent upon serotonergic input from the midbrain raphe nuclei.

Like many other 5-HT₄ agonists (e.g., buspirone, ipsapirone), NAN-190 is an antagonist which binds to 5-HT₄ receptors. However, unlike 5-HT₄ agonists, NAN-190 antagonized the discriminative effects of 8-COH DPAT (Glenon et al., Drug Dev. Res. 16: 335-343, 1989). We have now further evaluated NAN-190's effects on baseline behavior in a 5-HT₄ antagonist. In hypothermic assays, mice were treated with 10 mg/kg 8-COH DPAT to give a temperature drop of 6.0±0.9°F. NAN-190 (gift of R.A. Glenon) failed to antagonize this effect of 8-COH DPAT, but did itself depress body temperature.

To more specifically investigate 5-HT₄ mechanisms, we measured its effects on 5-HT₄ neuron firing rates. Dorsal raphe 5-HT₄ neurons identified according to Agahiyanian et al. (JPET 137: 178, 1970) were depressed by 8-COH DPAT (ED₅₀, 1.6 μg/kg). Again, NAN-190 did not reverse the depressant effects of 8-COH DPAT, but did itself depress firing rates with a potency (ED₅₀, 11 μg/kg) similar to that for buspirone, ipsapirone, and gepirone (7-15 μg/kg). It is concluded that behavioral antagonism by NAN-190 is unlikely to result from blockade of presynaptic 5-HT₄ receptors, but could result from antagonism of postsynaptic 5-HT₄ receptors.

5-HT₁A AND OTHER SHT RECEPTOR SUBTYPES


Like many other 5-HT₄ agonists (e.g., buspirone, ipsapirone), NAN-190 is an antagonist which binds to 5-HT₄ receptors. However, unlike 5-HT₄ agonists, NAN-190 antagonized the discriminative effects of 8-COH DPAT (Glenon et al., Drug Dev. Res. 16: 335-343, 1989). We have now further evaluated NAN-190's effects on baseline behavior in a 5-HT₄ antagonist. In hypothermic assays, mice were treated with 10 mg/kg 8-COH DPAT to give a temperature drop of 6.0±0.9°F. NAN-190 (gift of R.A. Glenon) failed to antagonize this effect of 8-COH DPAT, but did itself depress body temperature.

To more specifically investigate 5-HT₄ mechanisms, we measured its effects on 5-HT₄ neuron firing rates. Dorsal raphe 5-HT₄ neurons identified according to Agahiyanian et al. (JPET 137: 178, 1970) were depressed by 8-COH DPAT (ED₅₀, 1.6 μg/kg). Again, NAN-190 did not reverse the depressant effects of 8-COH DPAT, but did itself depress firing rates with a potency (ED₅₀, 11 μg/kg) similar to that for buspirone, ipsapirone, and gepirone (7-15 μg/kg). It is concluded that behavioral antagonism by NAN-190 is unlikely to result from blockade of presynaptic 5-HT₄ receptors, but could result from antagonism of postsynaptic 5-HT₄ receptors.

5-HT₁A AND OTHER SHT RECEPTOR SUBTYPES


Like many other 5-HT₄ agonists (e.g., buspirone, ipsapirone), NAN-190 is an antagonist which binds to 5-HT₄ receptors. However, unlike 5-HT₄ agonists, NAN-190 antagonized the discriminative effects of 8-COH DPAT (Glenon et al., Drug Dev. Res. 16: 335-343, 1989). We have now further evaluated NAN-190's effects on baseline behavior in a 5-HT₄ antagonist. In hypothermic assays, mice were treated with 10 mg/kg 8-COH DPAT to give a temperature drop of 6.0±0.9°F. NAN-190 (gift of R.A. Glenon) failed to antagonize this effect of 8-COH DPAT, but did itself depress body temperature.

To more specifically investigate 5-HT₄ mechanisms, we measured its effects on 5-HT₄ neuron firing rates. Dorsal raphe 5-HT₄ neurons identified according to Agahiyanian et al. (JPET 137: 178, 1970) were depressed by 8-COH DPAT (ED₅₀, 1.6 μg/kg). Again, NAN-190 did not reverse the depressant effects of 8-COH DPAT, but did itself depress firing rates with a potency (ED₅₀, 11 μg/kg) similar to that for buspirone, ipsapirone, and gepirone (7-15 μg/kg). It is concluded that behavioral antagonism by NAN-190 is unlikely to result from blockade of presynaptic 5-HT₄ receptors, but could result from antagonism of postsynaptic 5-HT₄ receptors.

5-HT₁A AND OTHER SHT RECEPTOR SUBTYPES


Like many other 5-HT₄ agonists (e.g., buspirone, ipsapirone), NAN-190 is an antagonist which binds to 5-HT₄ receptors. However, unlike 5-HT₄ agonists, NAN-190 antagonized the discriminative effects of 8-COH DPAT (Glenon et al., Drug Dev. Res. 16: 335-343, 1989). We have now further evaluated NAN-190's effects on baseline behavior in a 5-HT₄ antagonist. In hypothermic assays, mice were treated with 10 mg/kg 8-COH DPAT to give a temperature drop of 6.0±0.9°F. NAN-190 (gift of R.A. Glenon) failed to antagonize this effect of 8-COH DPAT, but did itself depress body temperature.

To more specifically investigate 5-HT₄ mechanisms, we measured its effects on 5-HT₄ neuron firing rates. Dorsal raphe 5-HT₄ neurons identified according to Agahiyanian et al. (JPET 137: 178, 1970) were depressed by 8-COH DPAT (ED₅₀, 1.6 μg/kg). Again, NAN-190 did not reverse the depressant effects of 8-COH DPAT, but did itself depress firing rates with a potency (ED₅₀, 11 μg/kg) similar to that for buspirone, ipsapirone, and gepirone (7-15 μg/kg). It is concluded that behavioral antagonism by NAN-190 is unlikely to result from blockade of presynaptic 5-HT₄ receptors, but could result from antagonism of postsynaptic 5-HT₄ receptors.
427.5

Using cortical slices in vitro, we have previously reported that both the 5-HT 1A agonist 8-OH-DPAT, and 5-HT reuptake inhibitors block the 5-HT releasing effects of either 5-HT 1A agonists (e.g., TMPP, RU 24966), or 5-HT toxins (e.g., p-CAMPH, MDMA) thus providing evidence for a functional interaction between 5-HT 1-receptor agonists with the 5-HT reuptake site (Galloway et al., this meeting). The present study examined potential interactions between 5-HT 1-receptor agonists and the 5-HT reuptake site in vivo. Fluoxetine (5 mg/kg, s.c.) a 5-HT reuptake inhibitor, completely blocked the ability of CP-93,129 (6 mg/kg, s.c.) to reduce endogenous 5-HT levels in both the medial prefrontal cortex (MPF) and the striatum (STN). Pretreatment with 8-OH-DPAT at 0.5 and 1.0 mg/kg resulted in a 40% and 70% reversal of the p-CAMPH depletion of serotonin 5-HT, respectively. (+)-MDMA (10 mg/kg, s.c., administered at 1, 2, 3, and 3.0 hrs before sac.) inhibited 5-HT synthesis in the anterior and posterior STN, MPF, nucleus accumbens (NAc), HC, and temporal cortex and decreased 5-HT levels (19 mg/kg, s.c., 3 hrs. before sac.) in the STN, MPF and HC. In contrast, MDMA did not decrease 5-HT in the NAc or OT and actually increased 5-HT synthesis in the OT. The 5-HT depleting effect of MDMA in the MPF was partially reversed by pretreatment with 8-OH-DPAT. Nomifensine (10 mg/kg, s.c.), a dopamine reuptake inhibitor, blocked the ability of 8-OH-DPAT to reverse MDMA toxicity in the MPF. The present study suggests an interaction between 8-OH-DPAT and the 5-HT reuptake site in vivo with a possible dopaminergic influence that is specific for DA projection fields. (Supported by MH-41227, DA-04120, and the State of Michigan DMH)
437.11 DEVELOPMENT OF SELECTIVE/POTENT 5-HT1A AGONISTS. R. K. RAGHUPATHY, L. HYDEBLE-FITZGERALD, M. TRITTEL, R. A. BURGESS, Department of Medicinal Chemistry, NV/CID, Research Triangle Park, NC 27709, and Department of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208.

5-NAN-190, 1-(2-methoxyphenyl)-4-[(4-(2-phthalimidobutyl)piperazine), has been previously reported to be a highly potent and selective antagonist of 5-HT1A receptors. We report here the synthesis and characterization of a series of 5-NAN-190 analogs. In a laboratory assay (Ki = 0.6 nM) 5-HT1A antagonist. (Glenm et al., Eur. J. Pharmacol., 1993, 234, 199-108).


P. maniculatus, the dormouse, displays both spontaneous and induced daily torpor bouts, assuming minimum body T of 15-20 C. There is evidence that brain serotonin may be involved in the initiation and homeostatic control of torpor. The serotonin synthesis markedly reduces the duration and depth of torpor (Pivornik and Axton, Lasing in the cold. pp. 323-39, 1986) while an increase in activity of the serotonergic and dopaminergic systems has been shown in the hypothalamus and SCN during daily torpor (Lin and Pivornik, Brain Behav. and Dev. 33:309-14, 1989). A certain percentage of deer will enter torpor under any circumstances (30-40%). We compared 3-HT receptor subtypes in dormice that readily enter into torpor (TP) and in non-torpor prone (NTP) animals. Deer mice were trapped in the wild and subjected to food rationing and low ambient temperature. They were sacrificed at 11 P.M. or at 11 A.M. Whole brain was assayed for 5HT1A and 5HT2 receptor differences using [3H]-OH-DPAT and [3H]-R-PIA respectively. Serotonin-1A receptor subtypes revealed both Vmax and Kd for BMY14800 (Bmax) of 5HT1A brain receptors was higher in both TP and NTP animals at 11 P.M. then at 11 A.M. Furthermore, at 11 P.M. 5HT1A receptor binding was significantly higher in TP animals compared to the NTP. No significant differences were found in TP and NTP animals indicating that serotonin receptor subtypes may be involved in the modulation of torpor in this species.


DRG neurons exhibit 3 distinct membrane responses to 5-HT: depolarization with increased or decreased input resistance (Rin) and hyperpolarization. Depolarization with increased Rin is mediated by 5-HT3 receptors while depolarization with decreased Rin is mediated by 5-HT3 receptors (Brain Research, 511, 71-79, 1990). We have now estimated reversal potentials (Erev) and their effect on cell firing in the presence and absence of 5-HT. In cells depolarized by 10 mM K+ with increased Rin if the Erev was -105.7±11.8 mV (N=6) in 2.5 mM K+, and the Erev for the 5-HT3 antagonist alpha-Methyl-tryptophan was "473±60 mV (N=8). Increases in the extracellular K+ produced a linear shift in the Erev as predicted by the Nernst equation, indicating the 5-HT3 response is mediated by a decrease in K+ conductance. In cells depolarized by 1 mM 5-HT with decreased Rin, the Rin was "30.2±1.8 mV (N=5) and with the selective 5-HT3 antagonist 2-methyl-5-HT3 (IC50=0.1 mM) the Erev was "32 mV (N=2). K+ electrodes did not alter this response. In N=4, free solution, both agonists produced hyperpolarization, indicating this response involves increased Na+ conductance. The negative Rg suggests that an increased K+ conductance also occurs. In 18 cells 5-HT hyperpolarized a decrease Rin. One mM 5-HT achieved an Erev averaging "462±56 mV (N=9). This was mimicked by the 5-HT1 agonist, carbachol and ethyl isopropyl (Emax = 4.2±0.7 mV, 1 mM, N=5). This response was not blocked by ketanserin or ICS 205-938, but 100 mM methoxethanilol (a broad 5-HT/5-HT2 antagonist) produced a parallel rightward shift in the 5-HT concentration response curve. These data suggest that a 5-HT-2 receptor mediates hyperpolarization in rat DRG cells.


We have shown previously that serotonin (5-HT) both depolarizes pyramidal neurons and excites firing of a subpopulation of interneurons in the piriform cortex. The 5-HT1C antagonist ritanserin blocked the activation of 5-HT3 receptors but not that of 5-HT1 receptors. We have now investigated the depolarization of pyramidal cells. Because ritanserin has a nearly 10 fold higher affinity for the 5-HT3 receptor than for the 5-HT1C receptor (Hoyer, 1990), we used ritanserin to block the 5-HT1 receptors and used 5-HT to activate 5-HT3 receptors.

5-HT1C antagonists in the piriform cortex indicate that 5-HT activates 5-HT1C receptors on pyramidal and interneurons. 5-HT activates 5-HT1C receptors on pyramidal and interneurons. The activation of 5-HT on interneurons produces a depolarization of 100-150 mV. The 5-HT3 receptor is activated by depolarizing current pulse; this effect of 5-HT is reversed by 100 nM. Tetrodotoxin is blocked by 100 nM TTX. These findings indicate that 5-HT in the piriform cortex acts via 5-HT1C receptors on pyramidal neurons and 5-HT1C receptors on interneurons. Supported by PHS Grants GM07534 and MH17871.


Destabilization of H-R-G complexes was used as a model to study initial events of signal transduction. A crucial step is the allosteric interaction between hormone receptor sites and G proteins. This phenomenon was examined in the adenylate cyclase system of beta2-adrenoceptors, the adenosine A2 (AD A2), the agonist (R)-3-phenylisopropyladenosine (R-PIA), and the 5-HTA receptor agonist (H)-BMY 14800, with both agonist (H)-BMY 14800 and the partial agonist (H)-BMY 7378. Progressive occupancy of the G protein by either agonist (H)-BMY 14800 or guanosine 5’-O-(3-thiotriphosphate) (GTPS) resulted in a concomitant decrease in the G protein density, with GTPS resulting in a lower concentration for all parameters. Parameters of concentration-effect curves (IC50, slope index and Emax) were determined for GTPS and GTPS at different occupancy levels of the receptor sites. For all drugs, IC50 values increased with increasing receptor occupancy, while maximal state inhibition of (H)-R-PIA or (H)-BMY-DPAT binding was independent of receptor occupancy. Both GU inhibition lower IC50 values on the 3HT1A antagonists than those on the AD A2 agonist. Thus, (H)-BMY 7378 density OH-DPAT and (H)-BMY 7378 binding, suggesting efficacy independence in this event of receptor/G-protein coupling. We propose that characteristics of destabilization of the H-R-G ternary complex to significant difference was seen at 11 type and degree of receptor occupancy, but are independent of drug efficacy. (USPHS GM 54852)
147.17

LACK OF 5-HT INHIBITION OF ADENYLYL CYCLASE IN BOSAL RAPHE OF MALE AND FEMALE RATS. W.P. Clarke1, E. Soto1, M. Nazario1, B. Marqués1, and M. Garcia2. 1Department of Pharmacology and 2Department of Psychiatry, University of Puerto Rico, Carolina, PR 00984.

In hypogonadal or castrated, 5-HT(1A) receptor subtypes are linked to at least two distinct responses: 1) inhibition of pyramidal cells, mediated by the opening of a second messenger K+ channel (Andrade et al., Science, 234:1251, 1986) and 2) inhibition of cerebral vasomotor activity (Rakieten, Nature, 307:281, 1990). We have used rat and mouse vas deferens to demonstrate sexual dimorphism in the expression of these responses. Thus, the inhibitory response to 5-HT was observed in the presence of theophylline in both male and female rat vas deferens, whereas in the mouse, the inhibitory effect was observed only in females. This finding suggests that the expression of this response is under the control of sex steroids and has important implications for the role of 5-HT in the control of vasomotor function.

147.18

INITIAL CHARACTERIZATION OF 5-HT RECEPTOR PRESENCE IN ASCARIS SUUM MUSCLE. J. Williams and A. Sharakhov, Department of Biological Sciences, University of North Texas, Denton, TX 76203.

Ascaris suum is a parasitic nematode which inhabits the upper third of the small intestine of porcine species. During n-feeding periods of the host A. suum is dependent upon muscle glycogen stores. 5-hydroxytryptamine (5-HT) initiates glycogenolysis in A. suum muscle via an increase in cAMP levels. Functionally, the 5-HT receptor has been linked to the cAMP system, which is activated by the phospholipidinolysis system, and the 5-HT receptor to ligand gated ion channels. Although ligand binding to the A. suum 5-HT receptor appears to initiate glycogenolysis through the cAMP system and not through the phospholipidinolysis system, binding assays with [3H]-HT, used to identify 5-HT1 and 5-HT2 sites, and 5-HT receptor subtypes have strong positive results. In contrast, assays using [3H]-SD, which has affinity for both 5-HT1 and 5-HT2 sites, and memanin as a competitor showed binding sites to be present to a much higher degree. When 5-HT was used as the competitor for [3H]-SD, once again minimal binding was present. Based on the results of the [3H]-HT and [3H]-SD binding assays, it was anticipated that binding of [3H]-metanis, a specific 5-HT2 ligand, would be robust. However, a low level of [3H]-metanis sites was found. This initial pharmacological profile may indicate the presence in A. suum muscle of several 5-HT receptor subtypes similar to mammalian 5-HT receptor subtypes and/or a novel non-5-HT1 receptor which functions through the cAMP system. Further comparisons of the A. suum 5-HT receptor to the mammalian 5-HT receptor subtypes are being carried out.

437.19

MDL 100,907, (+)-(2,3-dimethylphenyl)-1-[2-(4-fluorophenethyl)-4-piperidinemethanol], a potent, chiral, 5-HT receptor antagonist. H. Dudley, A. Ogden, K. Gatt, J. Bedell, and J. Kehne, Merrell Dow Research Institute, Cincinnati, OH 45225.

The racemic compound MDL 11,939 (a-phenyl-1-(2-fluorobenzyl)piperidinemethanol) has been reported previously as a potent and selective 5-HT receptor antagonist (Dudley et al. Drug Dev. Research 13:29, 1988). Synthesis of the enantiomers MDL 28,231, the (+)-isomer, and MDL 28,727, the (-)-isomer showed that the potent 5-HT receptor antagonism was associated with the (+)-isomer. MDL 28,727 had a value at rat cortical 5-HT receptor of 4.2 nM compared to 190 nM and 10 nM for MDL 28,231 and MDL 11,939, respectively. Further investigation of possible substituents on both aryl nuclei to increase potency resulted in the racemic MDL 100,151 (a-phenyl-1-[2-(4-fluorophenethyl)-4-piperidinemethanol], which was resolved into MDL 100,907, the (+)-isomer and MDL 100,009, the (-)-isomer. In initial studies DLD-1 samples in rat cortical membranes, MDL 100,151, MDL 100,907 and MDL 100,009 had IC50 values of 1 nM, 0.4 nM and 12 nM respectively. MDL 100,907 blocked 5-hydroxy-N,N-dimethyltryptamine-induced head twitch in mice with an ED50 of 0.03 mg/kg, i.p. MDL 100,907 was 130-fold selective for the 5-HT receptor over the 5-HT3 receptor. In conclusion, MDL 100,907 is a stereospecific, potent and selective 5-HT receptor antagonist.

437.20

DIFFERENTIATION OF 8-OH-DAFAT AND IPIPAFARINE IN RAT MODELS OF 5-HT RECEPTOR FUNCTION. R. de Vries, B. Schielrick, J. M. Grégoire, R. Horsthemke, T. Clasen. Institute for Neurobiology, Troponwerke GmbH & Co. KG, Berliner Str. 156, 5000 Köln 80, F.R.G.

8-OH-DAFAT (D) and ipsapirone (I) bind 5-HT3 receptors located presynaptically (PREsyn) in the raphe nuclei and postsynaptically (POSTsyn) predominantly in the limbic system. In the forskolin stimulated adenylate cyclase model, the 5-HT3 behavioral syndrome test, and the circling test (after unilateral application of 5-HT on the substantia nigra), three models of POSTsyn 5-HT3 receptor activation, D and I are characterized as full and partial agonists, respectively. D completely suppresses rat cortical 5-HT cell firing in slice preparations and in vivo; suggesting that both compounds are PREsyn 5-HT3 agonists. In drug discrimination (D cue) and hypoactivity, models which presumably involve PRE and POSTsyn 5-HT3 receptors, D and I are full agonists. To conclude, D appears that D is a full agonist at PRE- and POSTsyn 5-HT3 receptors; whereas I is a full and partial agonist at PRE- and POSTsyn receptors, respectively. However, the finding that (I) i, like D, completely generalizes to the D cue when applied bilaterally in the dorsal hippocampus, and (2) both I and D retain full agonistic activity in the hyperthermia model after 5,7-DHT induced lesion of the brain 5-HT system, suggest that there are brain regions where both compounds are full agonists at POSTsyn 5-HT3 receptors.

438.1


Mice and rats made physically dependent on ethanol have increased numbers of brain peripheral-type benzodiazepine receptors (PBR). To determine if this change was measured in C57BL/6J mice which consumed 3.5% ethanol (nondependent) or 7% ethanol (physically dependent). Binding of [3H]Ro5-4864 to olfactory bulb, pons-mesencephalon, cerebellum, and anterior and posterior cerebellum membranes indicated increased PBR in posterior cerebellum of physically dependent mice and no changes in other regions. Nondependent mice had reduced PBR in olfactory bulbs with no change in other regions. Cardiac and spleen PBR were not altered, although organ weights decreased. Thymus PBR increased in nondependent mice and tended to increase in dependent mice, despite pronounced thymic involution. K+ values were not significantly altered by chronic ethanol exposure. These results suggest that physical dependence upregulates PBR in posterior cerebellum brain regions and "normalizes" changes in olfactory bulb PBR found in nondependent mice. Whether alterations in brain PBR function accompany chronic ethanol exposure requires further study. (NIAAA grant AA07251.)

438.2


Abecarnil (ABC) is a non-selective anxiolytic -beta-carboline (Stephens, D.N. et al., JEP 253: 1990). For the investigation of tolerance phenomenon we used the antagonist properties of ABC (Turks, L. et al., JEP 253: 1990). Repeated treatment with ABC (15 mg/kg, twice daily for 12 days) led to a slight reduction in its potency as antagonism of the induced antagonist (PTZ) test in mice. The serum threshold for 5 mg ABC/kg, 30 min declined from 152 mg PTZ/kg (acute) to 122 mg PTZ/kg (subchronic). For short term tolerance, the PTZ seizure duration was determined following ABC, 2 mg/kg, 30 min before administration, between 30 and 310 min. To determine the degree of benzodiazepine receptor occupation by ABC the animal received [3H]-lorazepam, 7.4 MBq/mg, 30 min before decapitation. Immediately following the PTZ test the animal was decapitated. Blood was collected for HPLC estimation of ABC, and the forebrain homogenized in cold buffer, and aliquots filtered for determination of bound radioligand. The time course of this parameters were measured in parallel at peak values at 40 min. However, when the correlation of PTZ antagonism and benzodiazepine receptor occupation were plotted for all time points it became evident that at the same degree of occupancy ABC was clearly more effective in the early phase (30-40 min) than in the second, elimination phase. This acute potency shift was caused to that followings Abacarnil. This 'bystander's' effect may be useful for the prediction of tolerance development.
428.3

**Psychological and Gender Influences on the Stress-Induced Decrease in Renal Peripheral Benzodiazepine Receptors in Rat, R.C. Dragan, A.P. Stringer, and P.V. Holmes. Schrier Research Laboratory, Dept. of Psychology, Bacon University, Providence, RI 02912.**

Earlier research has shown that exposure to environmental stress results in changes in the density of peripheral benzodiazepine receptors (PBR) in both central nervous system (CNS) and peripheral tissues. We have previously shown that these changes are due to the characteristic physical parameters necessary for these changes, however, the psychological dimensions of stress or gender influences have been less clear.

We now report that controllability and predictability of tailstock stress do not result in a modification of the stress-induced changes in renal PBR. Further psychological stress alone, using a conditioned fear paradigm, does not produce a change in the binding of 

$[3H]Diazepam$ to PBR in comparison to controls.

However, administration of an anesthetic dose of sodium pentobarbital (60mg/kg, i.p.) prior to the stress completely blocks the decrease in PBR indicating the importance of consciousness during stress. Finally, we also report gender differences in the response of renal PBR to inescapable stress. Female rats show an attenuated decrease in PBR (23%) as compared to male rats (54%). These data suggest both neural and hormonal modulation of the stress-induced alterations in renal PBR.

SUPPORTED BY: NIMH grants R01-44034-01A1 & an Alfred P. Sloan Research Fellowship to Robert C. Dragan.

428.5

**Homologous and Heterologous Uncoupling of the GABA$_A$ Receptor Complex by GABA, 3a-0H-DHP, Flurazepam and Pentobarbital in Culture.** L. Kriedman, J.C. Bains, and D.B. Far. Dept. Anatomy & Cell Biology, BNIU, Health Sciences Center at Brooklyn, NY 11203.

We have previously shown that chronic treatment with GABA, certain steroids, benzodiazepines, or barbiturates produces a reduction in the allosteric coupling between the benzodiazepine recognition site and GABA binding to the GABA$_A$ receptor, which is referred to as "uncoupling." Here we describe homologous and heterologous uncoupling induced by chronic (88) exposure of neuronal cultures to GABA (11mM, 5-phenylpentadione (3a-0H-DHP), flurazepam (50mM), or pentobarbital (PBO) (200mM). Allosteric interactions were measured by the effect of cGMP 3a-0H-DHP, 500mM pentobarbital, or 10mM GABA on reversible binding of 0H-flunitrazepam binding by pentobarbital and 3a-0H-DHP, potentiation by GABA was only partially reduced (by 40%). Chronic GABA treatment greatly reduced (by 90%) PB-simulation of 0H-flunitrazepam binding, while steroid-stimulated 0H-flunitrazepam binding was unaffected. In contrast, chronic flurazepam treatment reduced the potentiation produced by all 3 compounds by a similar degree. Finally, pentobarbital exposure decreased both PB and GABA stimulation of 0H-flunitrazepam binding, but only if the presence of 0H-flunitrazepam binding. Coincubation of cultures with 3a-0H-DHP and SR-95531, a selective GABA$_A$, antagonized, the steroid-induced decrease of GABA potentiation but did not prevent the loss of 3a-0H-DHP stimulation of 0H-flunitrazepam binding.

These results suggest the existence of multiple modes of uncoupling which are most likely mediated through different sites.

428.6

**Effect of Desipramine (DMI) on High Frequency Neurotransmission (HN) and Receptor Inhibition (RI) in Rat Hippocampal Slice.** F.A. Dejka, J.H. Corpron, and G.B. Ruiz. Organon Int, CNS Pharmacology Dept., PO Box 30, 5755 BV Or, The Netherlands.

Tricyclic antidepressants are known to have specific acute interactions with several neurotransmitter systems in rat brain. Some adaptive changes to chronic antidepressant treatment (rather than the acute pharmacological properties of the drugs, seem to contribute to the understanding of their mechanisms) of action. In the present study, desipramine (DMI) was used by acute treatment in vitro (10uM), and single dose and long-term treatment in vivo (10mg/kg) on excitatory and inhibitory neurotransmission in the hippocampal slice. We recorded ortho- and antidromically evoked field potentials in stratum radiatum (EOGP) and pyramidal (PS) regions of the CA1 and CA3 sectors of the hippocampal slice. We recorded ortho- and antidromically evoked field potentials in stratum radiatum (EOGP) and pyramidal (PS) regions of the CA1 and CA3 sectors of the hippocampal slice. We recorded ortho- and antidromically evoked field potentials in stratum radiatum (EOGP) and pyramidal (PS) regions of the CA1 and CA3 sectors of the hippocampal slice. We recorded ortho- and antidromically evoked field potentials in stratum radiatum (EOGP) and pyramidal (PS) regions of the CA1 and CA3 sectors of the hippocampal slice. We recorded ortho- and antidromically evoked field potentials in stratum radiatum (EOGP) and pyramidal (PS) regions of the CA1 and CA3 sectors of the hippocampal slice. We recorded ortho- and antidromically evoked field potentials in stratum radiatum (EOGP) and pyramidal (PS) regions of the CA1 and CA3 sectors of the hippocampal slice. We recorded ortho- and antidromically evoked field potentials in stratum radiatum (EOGP) and pyramidal (PS) regions of the CA1 and CA3 sectors of the hippocampal slice. We recorded ortho- and antidromically evoked field potentials in stratum radiatum (EOGP) and pyramidal (PS) regions of the CA1 and CA3 sectors of the hippocampal slice.
428.11 COMPARISON OF AGONIST AND ANTAGONIST COMPETITION FOR (3H)HRAULOSCINE (RAU) BINDING TO α2 ADRENERGIC RECEPTORS (ADAR) OF INTACT CHINESE HAMSTER Ovary (CHO) CELLS AND ISOLATED MEMBRANES. P.E. Shreve, M.L. Towns, and D.B. Bylund. Dept of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE 68198.

α2-adrenergic agonists exhibit markedly lower binding affinities for BAR intact cells as compared to isolated membranes. In contrast, BAR antagonists have similar affinities for BAR intact cells and membranes. This phenomenon has been similarly observed for α2-adrenergic and muscarinic receptor systems (J. Pharmacol. Exp. Ther. 251(1):63-70, 1989). These changes in binding properties may be related to differences in regulation of desensitization.

The purpose of this study was to compare the binding affinities of agonists and antagonists to intact cells and isolated membranes of CHO cells which have been stimulated and harvested the same day. Pharmacological studies indicated that (3H)HRAU, an α2 agonist, exhibited high affinity for both intact CHO cells and CHO membranes with Kd = 1.3 and 0.53 nM, respectively. Noncompetitive inhibitors of the α2 receptor were tested on intact membranes. A 3-hr treatment of cells with 3 μM olanzapine inhibited α2AR binding in intact CHO membranes while not affecting its affinity in CHO membranes. This difference in agonist binding affinity between intact cells and isolated membranes is similar to that observed for other receptor systems and may play a role in the α2 receptor-induced desensitization. This desensitization was supported by NIH grant GM07664.


We have previously used autoradiography to demonstrate that chronic reserpine administration increases β-adrenergic receptor binding in many brain regions (FASEB J. 4: A499, 1990). In this study we examined for reserpine-induced changes in β-stimulated cAMP production, comparing two general cortical regions. Rates were measured for 15 days with reserpine protection 0.5 mg/kg days 1 & 2, and 0.25 mg/kg thereafter. Control cultures were sacrificed 24 hrs after the last dose and brains were dissected sagitally. Half was frozen for binding analysis, half used for cAMP analysis. The cortex was dissected free and divided by a knife cut parallel to the rhinal sulcus into an "upper" region (essentially neocortex, essentially limbic cortex (rhinal cortex and amygdala). 350 μl slices were prepared and stimulated with various agonists; cAMP levels were measured by RIA. Stimulation (3-6 h) was seen with isoproterenol (NE) and Forskolin, but not 6-FN alone. The stimulation by NE, ISO, and 6-FN alone was not forskolin, in which instance was less in the neocortical than in the limbic cortex. Regional differences support a difference in the efficiency of coupling of β-receptors to adenylyl cyclase in neocortex compared to limbic cortex.

428.13 EFFECTS OF REPEATED ELECTROCONVULSIVE SHOCK (ECS) ON SERTONIN-1A (5-HT-1A) RECEPTOR BINDING AND RECEPTOR MODULATED HYPOTHERMIA IN RAT. C.A. Stockmeier and G.A. Gudelsky. Departments of Psychiatry and Neurosciences. Case Western Reserve University, Cleveland, OH 44106.

5-HT-1A receptors may be involved in the mechanism of action of antidepressant drugs. Chronic antidepressant drugs or ECS attenuate 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)-induced hypothermia (Goodwin et al., 1987). Male rats were handled or received ECS for either one or ten days, and were killed two days later. Ten days of ECS decreased beta-adrenergic receptor binding in neocortex. In horizontal [3H]8-OH-DPAT binding in cortex or hippocampus was not affected by repeated ECS. In hypothalamus, ten days (but not one day) significantly decreased [3H]8-OH-DPAT binding. In addition, 48 hr following 10 days of ECS, the hypothermic response to 8-OH-DPAT (0.1 mg/kg, sc) was attenuated compared with handling controls. The hypothermic response to 8-OH-DPAT was returned to normal at 14 days after the last of ten treatments. A single treatment with ECS did affect the hypothermic response to 8-OH-DPAT. The ECS-induced decrease in [3H]8-OH-DPAT binding in the hypothalamus was reversed in the ECS-resistant strain of the intact rats. The decrease in the response to 8-OH-DPAT was also reversed by meal treatments. Supported by NS32532 and MH41684.
428.15  

To determine if repeated administration of antidepressants to rats regulate 5-HT uptake sites, the binding of [PHCN-111] was measured using quantitative autoradiography. Under our assay conditions, this radioligand binds specifically, and with high affinity, to 5-HT uptake sites (Brain Res. 667-1994). All antidepressants tested were administered for 21 days except clomipramine (14 days) and were given i.p. except for deprenyl (s.c.). The drugs used were: the 5-HT uptake inhibitors sertaline (5mg/kg, daily) and the serotonin reuptake inhibitor clomipramine uptake inhibi-
tor protriptyline (15mg/kg, twice daily); the 5-HT2 receptor antagonist mianserin (10mg/kg, twice daily); the non-selective MAO inhibitor phenel-
 zinc (5mg/kg, twice daily); and the type B MAO inhibitor deprenyl (0.25 mg/kg, daily). Rats were killed 18 hrs after the last injection of drug. All structures examined were from brain slices taken at the level of plate 30 of the atlas of Paxinos and Watson (1986). Administration of clomipramine or protriptyline had no effect on [PHCN-111] binding in any brain region exa-
mined. Treatment with sertaline, phenelzine, deprenyl or mianserin caused some regionally specific alterations in the number of binding sites, but the effects were modest in size (10-20%) and no common effects were seen. Thus, no consistent regulatory effects associated with 5-HT uptake are pro-
duced by antidepressant drugs. (Supported by research funds from the Department of Veterans Affairs.)

428.16  
**LONG-TERM BUT NOT SHORT-TERM LITHIUM TREATMENT POTENTIALS m-CPP INDUCED CHANGES IN Plasma PROLACTIN AND CORTICOSTERONE BUT NOT GROWTH HORMONE LEVELS IN RATS.** C.S. Aulakh, J. Hill, N.A. Garrett and D.L. Murphy. Lab. of Clinical Sciences, NIMH, Bethesda, MD 20892

The lithium ion is effective clinically for the treatment of acute manic illness, prophylaxis in manic depressive bipolar and unipolar depressive disorders, and in conjunction with antidepressant drugs for the treatment of resistant depressive illnesses (DeMONTIGNY et al. 1980, Prin et al. 1988, Price et al. 1990). The therapeutic effects of lithium contain a spectrum of psychopharmacological mechanisms regulating prolactin, corticosterone and growth hormone secretion following long-term lithium treatment in rats. Male Wistar rats were given rat chow containing lithium carbonate (1.65g/kg) for 28 days. Saline or various doses of m-CPP were injected intraperitoneally (11:31:35 a.m.) at least 48 hours after the animals were implanted in the blood vessel; blood samples were drawn 30 minutes after saline or m-CPP injection. Long-term (21-23 days) lithium treatment potentiated m-CPP-induced increases in plasma prolactin and corticosterone levels but did not have any effect on m-CPP-induced decreases in plasma growth hormone levels. Short-term (2-4 days) lithium treatment attenuated m-CPP's effect on plasma corticosterone levels without any effect on plasma prolactin or growth hormone levels. These findings document an enhancement of some aspects of brain 5-HT function following long-term lithium treatment and support other data suggesting that lithium's serotonergic actions may be relevant to its therapeutic efficacy.

**GABA<sub>ß</sub> RECEPTORS**

429.1  
**MODULATION OF INHIBITORY SYNAPTIC TRANSMISSION IN HIPPOCAMPAL SLICES BY ACTIVATION OF PRESYNAPTIC GABA<sub>ß</sub>-RECEPTOR**: D.J. Stanfield and H.Y. Wheel. Department of Neurophysiology, University of Southampton, Bassett Crescent East, Southampton SO9 0TU, UK. (Supported by the Brain Research Association, UK.)

CA1 pyramidal cell responses to paired stimuli were recorded extracellularly from the rat hippocampal slice preparation. The inhibition produced by the initial (conditioning) stimulus was quantified by measuring the response to the second (test) stimulus, and comparing it to the response to the initial stimulus. Experiments were performed to measure the effect of interpulse interval on the inhibition obtained. Orthodromically evoked response pairs for control slices (n=18) show inhibition at short interpulse intervals (0-20msec) and also at longer intervals (200-400msec), whereas antidromically evoked response pairs show no inhibition at these intervals. As has been previously reported, antagonists of GABA<sub>ß</sub> receptors (bicuculline 10mM) enhanced both responses, with a reduction in paired pulse inhibition (measured as the ratio of test to conditioning responses) at short intervals but increased inhibition at longer interpulse intervals (n=7).

Antagonists of the GABA<sub>ß</sub> receptor (phaclofen 1mM) had little effect on the magnitude of the reduction of the test response at long interpulse intervals, thus reducing paired pulse inhibition (n=7). When the GABA<sub>ß</sub> antagonist baclofen (1mM) reduced both responses whilst antagonizing paired pulse inhibition (n=7). Some proportion of the decrease in paired pulse inhibition can be attributed to the reduction in conditioning response (to 35% of control values). However, extrapolation from control experiments on the effects of changes in conditioning response amplitude, produced by varying stimulus intensities, would lead to the prediction of a smaller decrease in late inhibition. This leaves open the possibility that direct action of baclofen on presynaptic GABA<sub>ß</sub> receptors, located on inhibitory inter-

429.2  
**2-HYDROXY-SACLOFEN REDUCES THE DEPRESSION OF IPSCS INDUCED BY A Variety of STIMULATION Patterns IN Rats**. C.H. Darie* and G.L. Collins-GR. (SPON: Brain Research Association) Department of Pharmacology, University of Bristol, Bristol, BS8 1TD, UK.

Recently we reported that at an interstimulus interval of 100 ms paired-pulse depression of monosynaptic IPSCS was abolished by the GABA<sub>ß</sub> receptor an-
tagont 2-hydroxy-saclofen (Davies et al., 1990). We have now examined the effect of 2-hydroxy-saclofen on a wide range of intervals (25-2500 ms) as well as its effect on more complex stimulation paradigms. In the presence of D-AP5 (40-80m) and CNQX (20m) stimulation in striatum evoked monosynaptic IPSCS. 2-hydroxy-saclofen (400mM) abolished paired-pulse depression of IPSCS at all interstimulus intervals tested. Complete block of the depression induced by stimulus trains (5-10 Hz for 1 s) was also achieved although at frequen-
cies of >10 Hz 2-hydroxy-saclofen only partially reversed the depression of IPSCSs. We have also tested 2-hydroxy-saclofen on IPSCS evoked by primed-pulse (e.g., 2 shocks of 20-100 Hz preceded 140-170 ms by a single shock) and patterned-burst (e.g., 5 bursts of 4 shocks of 100 Hz delivered at 200 ms intervals) stimulation paradigms. In all cases 2-hydroxy-saclofen partially reversed the depression of synaptic inhibition.

429.3  
**BICUCULINE-RESISTANT GABA<sub>ß</sub> MEDIATED EVENTS DISCLOSED BY THE GABA<sub>ß</sub> ANTAGONIST 2-HYDROXY-

429.4  

We studied the actions of the GABA<sub>ß</sub> receptor antagonist 2-
hydroxy-saclofen (2-OH-S) and baclofen (BAC) on the GABA<sub>ß</sub> mediated events and the effects of these agents on the motor output of the hippocampus. The GABA<sub>ß</sub> receptor antagonist baclofen (BAC) hyperpolarizes pyramidal neuront by activating postsynaptic GABA<sub>ß</sub> receptors that mediate the loss IPSC recorded from these neurons, and depresses synaptic potentials evoked by stimulation of Schaffer collateral/commissural fibers, presumably by activating presynaptic GABA<sub>ß</sub> receptors. Lower concentrations of 2-OH-S (50-100mM) antagonized the postsynaptic action of BAC and reduced the amplitude of the late IPSC. Lower concentrations of 2-OH-S (200-500mM) were effective. 200mM 2-OH-S inhibited the dose-response curve for depression of IPSCS by BAC to the right by a factor of 4, indicating a pA<sub>ß</sub> value of 4 for 2-
OH-S at presynaptic GABA<sub>ß</sub> receptors. GABA<sub>ß</sub> receptors did not change excitatory transmission or postsynaptic membrane properties. Phaclofen (0.3-
mM) also blocked the late IPSC but had only a weak effect on presynaptic receptors. We conclude that 2-OH-S is a more potent antagonist than phaclofen at pre- and postsynaptic GABA<sub>ß</sub> receptors in the hippocampus. 2-OH-S and phaclofen may have a higher affinity for postsynaptic than presynaptic GABA<sub>ß</sub> receptor.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
429.5  
MECHANISMS OF BACLOFEN- AND OPIOID-INDUCED DISINHIBITION IN AREA CA1 OF RAT HIPPOCAMPUS*  

N.A. Lambert, E.H. Nirenberg, and T.J. Tevlin  
Department of Neurology, Northeastern University College of Medicine,  
Boston, MA 02111.  

The effects of (R)-HA-966, an agonist at N-methyl-D-aspartate (NMDA) receptors, and of the opioid receptor agonist, (R)-HA-966, on GABAergic neurons were examined in areas CA1 of the hippocampus of adult male rats.  

Electrical stimulation of the stratum radiatum, which depolarizes GABAergic neurons, and exposure to (R)-HA-966, but not (S)-HA-966, significantly decreased the amplitude of fast IPSPs recorded from these neurons.  

Furthermore, the effects of (R)-HA-966 were mimicked by (R)-HA-966, but not by the opioid receptor agonist, (R)-HA-966, which depolarizes GABAergic neurons.  

These results suggest that baclofen and opioids disinhibit pyramidal neurons in area CA1 of the rat hippocampus by activating receptors located on the terminals of GABAergic neurons, and by a mechanism that is insensitive to barium.  

Supported by the ONR.

429.6  
GABA RECEPTOR BINDING IN SYNAPTIC MEMBRANES THAT HAVE (ADULT RAT CEREBRAL) OR LACK (HIPPOCAMPAL CULTURE) A POSTSYNAPTIC POTASSIUM CONDUCTANCE COUPLED TO GABA RECEPTORS.  

N.I. Al-Ghais and J. Thalmann, Baylor College of Medicine, Houston, TX 77030.  

GABA receptors in our cultures lack coupling with K channels (Hablitz and Thalmann, 1989, v3, p982) from adult rat, or from hippocampi dissociated at postnatal day 1 and cultured 7 days. Scatchard analysis of saturation studies of a specific binding of (3H)-baclofen indicated a single binding site in either preparation, with an affinity in cultured neurons (KD=45-60nM) similar to that in adult cerebral cortex (KD=49+9nM). The ability of GABA antagonists to displace baclofen binding was similar in both preparations: In both, the IC50 for phaclofen was approximately 100nM for saclofen, 10nM. The regulation of binding by calcium and by GTPγS was also similar in that in either preparation binding was totally dependent upon Ca and could be completely suppressed by GTPγS (100uM). Thus all the GABA receptors in each membrane may be affected by Ca and coupled to G-proteins. Even though the coupling of receptors to K channels differs in the two preparations, we have thus far detected no differences in the receptors themselves.  

Supported by NIH grant NS21713.

429.7  
Excitatory amino acids: NMDA receptor glycine and polyamine sites  

Excitatory amino acid (EAA) receptors are classified into two classes, the N-methyl-D-aspartate (NMDA) and the non-NMDA receptors (AMPA and Kainate). Non-NMDA receptors are activated by numerous neurotransmitters including glutamate and GABA. The glycine site at the NMDA receptor is important for maintaining the sensitivity of the NMDA receptor to glutamate.  

The glycine site is a potential target for drug development as many diseases, such as epilepsy, are associated with increased activity of the NMDA receptor.  

We examined the glycine site at the NMDA receptor using a competitive binding assay. The glycine site was found to be critical for the activation of the NMDA receptor.  

We also investigated the role of polyamines in the activation of the NMDA receptor. Polyamines, such as spermine and spermidine, are known to modulate the activity of ionotropic glutamate receptors.  

Our results suggest that polyamines may play a role in the regulation of NMDA receptor function.  

Supported by NIH grant NS21713.

430.0  

ICI Pharmaceuticals Group, ICI Americas, Tarrytown, NY.  

We report here the comparison of the in vitro stereoselectivity of the glycine receptor for the (R)-enantiomer of HA-966 (1-hydroxy-3-amino-pyrrolidone-2) to the stereoselectivity we have observed in vivo.  

The glycine agonist site of the N-methyl-D-aspartate (NMDA) receptor in vivo shows stereoselectivity for the (R)-enantiomer of HA-966.  

We now confirm in vitro this stereoselectivity for the (R)-enantiomer. We also report that the (S)-enantiomer is more potent in vivo against NMDA and D-serine evoked cGMP in cerebellum. Although the glycine receptor is stereoselective for the (R)-HA-966, the (S)-enantiomer may be more potent in vivo against NMDA and D-serine evoked cGMP in cerebellum.  

These results suggest that the glycine site at the NMDA receptor is important for the stereoselectivity of the receptor.  

Supported by NIH grant NS21713.

430.2  
ANALYSIS OF ETHANOL (EOH) INTERACTION WITH GLYCINE POTENTIATION OF NMDA-ACtIVATED CURRENT.  

H.M. White, D.M. Levinson, R. W. Peoples, and F.E. Wright.  
Section of Electrophysiology, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852  

The amplitude of NMDA-activated ion current is reduced in the presence of intoxicating concentrations of EOH (5-100mM). We have investigated whether the inhibition of NMDA-activated current by EOH is due to an alteration of the interaction between glycine and its binding site on the receptor/channel complex.  

Experiments were carried out on cultures of mouse hippocampal neurons using whole-cell recording.  

We evaluated the effect of 50mM EOH on the amplitude of the NMDA-activated current at different added glycine concentrations. Inhibition by EOH was 33+4% at 3.5, 39+5% at 10, 43+5% at 20, and 43+4% at 100mM. The EC50 for glycine was similar in the absence and presence of EOH (31+1 vs 36+1mM, respectively).  

Reduction in EOH inhibition was significant only at 50mM glycine (p<0.01, paired t-test), while maximal potentiation of the NMDA current was observed at 1mM glycine. Thus, high concentrations of glycine reduced the effect of EOH on NMDA current.  

The effect of EOH on NMDA current was also studied in the presence of glycine in the bath and in the pipette.  

The results were similar in both conditions.  

Supported by NIH grant NS21713.

The results of this autoradiographic study of rat forebrain sites indicate that the glycine receptor has two sites: (a) a glycine-7-amino-substituted-cyclopropyl (ACBC) and (b) 7-chlorokynurenate (7CHLKYN) differentially modulates interactions at the [3H]CPP-binding site (7CHLKYN) vs [3H]CPP (ACBC) recognition site. Specificity analyses indicates that 7CHLKYN and ACBC induce an increase in the number of [3H]CPP binding sites (Bmax: 0.46 +/- 0.01 to 0.74 +/- 0.01 pmoles in the striatum radiolat CA1) without significant alteration the affinity. Although the Bmax increases for 7CHLKYN in ACBC, a linear Scatchard analysis along with a Hill number near unity suggests similar characteristics for both the basal sites and induced sites, hence the increase in [3H]CPP binding are observed throughout the hippocampus. In contrast, 7CHLKYN does not stimulate [3H]CPP binding in the hippocampus, specifically, stratum oriens and stratum radiatum of CA1 and CA3 as well as dentate molecular layer. More significantly, 7CHLKYN non-competitively inhibits the [3H]CPP binding enhancement by ACBC.

These findings suggest that ACBC and 7CHLKYN represent distinct classes of glycine antagonists acting at separate recognition sites, which differentially modulate interactions at the coupled NMDA antagonist recognition site labelled with [3H]CPP. The increase in [3H]CPP binding sites induced by ACBC may be a result of the unmasking of nascent sites or the conversion of NMDA agonist-prefering sites to antagonist-prefering sites.

430.5 INTERACTIONS BETWEEN POLYAMINES, GLYCINE AND GLUTAMATE AT THE N-METHYL-D-ASPARTATE RECEPTOR, R.K. N cysteine, R. Singer, M.J. Fruscione*, D.G. Lewis, S.R. Zolkin, Department of Psychiatry and Neuroscience, Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY 10461

The goal of our study was to determine mechanisms involved in the interactions between spermidine and the NMDA receptor. The effect of increasing spermidine concentration upon specific binding of [3H]MK-801 to well-washed, frozen-thawed synaptic membranes derived from rat forebrain was studied in the presence and absence of 7-chlorokynurenate (7CHLKYN) or [3H]2-amino-5-phosphonovaleric acid ([3H]AP5) with and without added L-glutamate and/or glycine. 7CHLKYN attenuates NMDA receptor functioning by acting as a competitive antagonist of the NMDA receptor-associated glycine site, and [3H]AP5 as a competitive antagonist at the glutamate (NMDA) site.

7CHLKYN attenuated [3H]MK-801 binding in the presence of spermidine alone or in the presence of L-glutamate, but not in the presence of glycine. [3H]AP5 (10mM) attenuated [3H]MK-801 binding in the presence of spermidine alone, but not in the presence of L-glutamate or glycine. [3H]AP5 (10mM) attenuated [3H]MK-801 binding in the presence of spermidine with glycine, but not in the presence of L-glutamate.

Our results suggest: 1. Spermidine-induced stimulation of NMDA-receptor functioning requires the presence of glycine, or alternatively, 7CHLKYN may function as an inverse agonist at the glycine site.

Support: USPHS DA-03833, Ritter Foundation (SRZ); USPHS MH-40661 (DCJ); Dept. of Psychiatry, AECOM (Dr. H.M. van Praag, Chairman).

430.6 IFENPRODIL BLOCK OF NMDA CHANNELS IS PARTIALLY GLYCINE DEPENDENT. P. Legender*, G. Westbrook, INSERM, Bordes, France and Votum Institute, Oregon Health Sciences Univ., Portland, OR

The vasodilator ifenprodil has recently been shown to antagonize NMDA receptor-mediated currents. These effects may be due to the interaction with NMDA receptors in several experimental preparations, but its mechanism of action remains unclear. It has been suggested that ifenprodil (IF) antagonizes NMDA receptors by an interaction with polyamines. Carter et al. (1989), however, have demonstrated that ifenprodil dose-dependently inhibits [3H]MK-801 binding to the NMDA receptor of both rat brain and aortic smooth muscle. Inhibition of [3H]MK-801 binding is reversed by the addition of glycine. These results indicate that ifenprodil may act on an IF-sensitive, glycine-insensitive, NMDA-sensitive population suggesting that in addition to IF's direct action at "active" and/or "inactive" NMDA receptors, IF may unmask a heterogeneity in the NMDA receptor population.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
430.9  
SPERMIN DEPOLARIZES THE RAT CORTICAL WEDGE AND POSITIVELY MODULATES SPONTANEOUS EPILEPTIFORM DISCHARGES.  
The polyamine spermine reportedly modulates binding of NMDA and glycine to allosteric receptor sites of the NMDA receptor complex. We examined functional responses in the rat cortical wedge preparation to spermine in Mg++ free buffer with TTX (0.5 μM). Spermine (1 μM-3 mM) induced slow dose-dependent repetitive depolarizations which were not blocked by NMDA, but the NMDA antagonist CPP, the AMPA/kainate/glycine antagonist DNQX, or ifenprodil (a reported ligand of both the polyamine and Glu receptor sites). In the absence of TTX, spermine (100 μM -1 mM) increased (57-178%) the rate of spontaneous epileptiform discharges (SED) in a dose- and time-dependent manner. Ifenprodil (30 μM) inhibited SED; neither spermine (100 μM-3 mM) nor glycine (1 mM) reversed this inhibition. The orotidine decarboxylase inhibitor, GPO (MDL 71725A) (100 μM, 2 hrs) did not inhibit SED. In conclusion, spermine showed functional responses in the rat cortical wedge model in both the presence and absence of TTX, with unclear mechanisms of action. This study did not indicate a competitive interaction between spermine and ifenprodil in direct depolarizations or SED. Activation of NMDA, kainate, or glycine recognition sites do not appear to be involved in spermine-induced depolarizations.

430.10  
POLYAMINE ANTAGONIST REDUCES NMDA-INDUCED DEPOLARIZATION, BUT ALSO REDUCES MAGNESIUM ANTAGONISM.  
We have demonstrated that Mg2+ loss potently antagonizes depolarizations evoked by NMDA in 10-15 day old rats than in adults. Endogenous polyamines regulate opening of the NMDA channel, and they are most abundant during development. The polyamine 1,10-dimethanodiaminocane (DA10) inhibits the enhancement of [3H]NMDA-001 binding produced by glutamate, glycine and spermine. We therefore used DA10 as a tool to determine whether polyamines regulate the sensitivity of the NMDA receptor to Mg2+. A grease-gap preparation was used to study depolarizing responses of CA1 hippocampal pyramidal cells to NMDA. In the absence of added Mg2+, DA10 (316 μM) noncompetitively antagonized NMDA-evoked depolarizations, but did not affect depolarizations evoked by AMPA. The effect of DA10 was significantly greater in preparations from adult than from preparations from 10-15 day old animals. This difference may be explained by the greater concentration of polyamine present in younger animals. DA10 also greatly reduced the effectiveness of 1 mM Mg2+ in antagonizing NMDA-evoked depolarizations. The effect of Mg2+ was reduced by 70% in preparations from adult animals and was virtually abolished in preparations from 10-15 day old rats. These results suggest that polyamines regulate not only the sensitivity of the NMDA receptor to agonist, but also its sensitivity to Mg2+. DA10 may act as a polyamine antagonist for the former action and as a polyamine agonist for the latter action. (Supported by NIH grant NS 16064.)

430.11  
BINDING OF [3H]IFENPRODIL TO THE POLYAMINE-SENSITIVE DOMAIN OF THE NMDA RECEPTOR:  
F.M. Reart, L. D. Mercer*, A.J. Searle* and B. Jarrott*, University of Melbourne, Clinical Pharmacology, Austin Hospital, Heidelberg, 3084, Australia.  
The NMDA receptor-ionophore complex consists of a number of interacting domains, including one for polyamines where ifenprodil binds to its non-competitive antagonist. Ifenprodil was iodinated with Na125I/chloramine-T, purified and its binding characterized. In cerebellar membranes, binding was saturable (Kd 14500, Rmax 5.5 pmol/mg protein) and to a single site. Specific binding was reversible, being dissociable with I2 and poorly dissociable with 1 μM ethylene glycol. Inhibition constants for spermine and spermidine were 15 and 44μM, respectively. Spermine inhibited 125I/ifenprodil binding with Kd 82.0715.  
In autoradiographic studies using slide-mounted sections, binding sites for 125I/ifenprodil were localized in several regions including anterior cingulate cortex, hippocampus and amygdala. Whilst the polyamine site of the NMDA receptor can be successfully labelled with 125I/ifenprodil (Spemaker et al., Eur. J. Pharmacol. 178, 249, 1990), the 125I/ligand is a rapid, convenient and inexpensive alternative.

430.12  
NMDA RECEPTOR BINDING PROPERTIES OF 5,7-DICHLOROKYNURENIC ACID: A RADIOLABELLED GLYCINE SITE ANTAGONIST.  
The kynurenic acid (KA) derivative, 5,7-dichlorokynurenic acid (5,7-DCKA) is a potent and selective inhibitor of strychnine-insensitive NMDA receptor binding and a potent inhibitor of the NMDA receptor in brain membranes (IC50 = 79 nM) whereas IC50 values were >100 μM at a variety of other glutamate-related and non-glutamate neurotransmitter receptors. 5,7-DCKA non-competitively antagonized the NMDA binding site, thereby preventing activation of a significant number of regions of rat cerebral slices. This antagonism was prevented by glycine or D-serine. Using methods described for [3H]glycine binding, we found that 5,7-DCKA was a competitive inhibitor in brain membranes with Kd = 69 nM and Bmax = 14.5 pmol/mg protein. A concentration of 10 μM 5,7-DCKA gave approximately 9000 dpm (total) and 3000 dpm (non-specific) and exhibited a pharmacological profile which was consistent with labeling of the strychnine-insensitive glycine binding site. IC50 values (μM) were: 5,7-DCKA (0.03), glycine (0.3), D-serine (0.6), 7-Ch-KA (0.5), KA (10), L-serine (27), HA-966 (49), and TCOOH (89). Binding was allosterically regulated by the glutamate recognition site; increased by NMDA, Asp, and Glu and inhibited by phosphonoamino acid antagonists. This ligand should be a valuable tool to probe the NMDA receptor complex.

430.13  
KYNURENIC ACID ANALOGUES AS POTENT AND SELECTIVE ANTAGONISTS AT THE GLYCINE SITE ON THE NMDA RECEPTOR.  
The N-methyl-D-aspartate (NMDA) receptor possesses two separate amino acid recognition sites, one for acidic amino acids such as glycine and NMDA and one for neutral amino acids such as glutamate. The broad spectrum excitatory amino acid antagonist kynurenic acid (KYN) has affinity for both sites, however substitution of Cl in the position 7 yields a compound (7-Ch-KYNA) with improved potency and selectivity as a glycine site antagonist (Kemp et al, Proc Natl Acad Sci, 85:6847, 1988). In a magnetic binding assay using P3 membranes from rat cortex and hippocampus, the affinity of 7-Ch-KYNA (IC50 = 0.56 μM) was improved in the analogues 5,7-dichlorokynurenic acid (IC50 = 0.2 μM) and 5,7-dichloroindole-2-carboxylic acid (IC50 = 0.005 μM). These new KYNA analogues retained good selectivity (> 2 orders of magnitude) versus other acidic amino acid binding sites.  
In a rat cortical slice assay and whole cell voltage-clamp recordings from rat cerebral cortical neurons, these compounds were selective, non-competitive antagonists of NMDA responses (respective IC50's of 3.0 and 0.41 μM in the cortical slice). Both compounds caused a complete flattening of the NMDA concentration-response curve, and their antagonism was reversed by co-application of glycine or D-serine, consistent with a selective action at the glycine site. The improved selectivity and affinity of these compounds for the glycine site on the NMDA receptor should make them useful as tools for further studies.

The present study used high-speed chronoamperometry to examine the effects of locally applied DOPamine and DAGO, two selective delta and mu2 receptor agonists respectively, on basal and event-related dopamine release in situ in the primate striatum (CA and nacc. accumbens). Male rats were anesthetized with either chloral hydrate or urethane, tracheostomized and placed in a stereotactic frame. Nafion-coated carbon fiber electrodes were inserted into either the pillared IP of the D1 dopamine receptor in the dopaminergic terminal area (e.g., 30-40 µm) with a tip separation of 200-300 µm and lowered to various depths into the nucleus accumbens (NACs) and, in some cases, into the basal ganglia, with the other electrode being either a DPFA or DAGO (1,10 or 100 mM). DOPamine concentrations were determined by applying the electrochemical method at a rate of 5 Hz, a +0.5V (vs. AgCl reference electrode). The effects of D1 receptor agonists on dopamine release were monitored for 180-300 sec following self-injection. In both cases, dopamine release was increased by 50% above baseline, compared to APDs, and this increase was observed to be due to DPBlock as it was not reversed by membrane hyperpolarization with amiloridine (0.01 or 0.05 mg/kg, i.v.). In conclusion, these results indicate that D1 DA receptor agonists do not exert electrophysiological effects on DA neuronal activity characterized by spontaneous activity of standard APDs, and suggests that either D1 receptor agonists might not be APDs or that D1 may have a different mechanism that may not be a necessary component of APD action (Supported by MN-40832, DA-04093 and the Michigan Department of Mental Health).

43.2 ELECTROPHYSIOLOGICAL STUDIES OF MIDBRAIN DOPAMINE NEURONS IN CULTURE. D.L. Cardozo Department of Neurobiology, and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

I have removed midbrain dopamine neurons from neonatal rats and routinely maintained them in dissociated cell culture for several months. This preparation has been confirmed by catecholamine fluorescence and by tyrosine hydroxylase immunocytochemistry. Living DA neurons have been identified for electrophysiological recording and recordings made with both high resistance and patch electrodes. The observed physiological properties of cultured DA neurons are consistent with results from in vivo and slice preparations. These include long-duration action potentials (2.5-4 mV); 1.5 Hz pacemaker-like firing activity; 450 ms slow depolarization and spontaneous excitation. In addition, DA cell firing was inhibited by applied DA or (+/-)-PPTP, a potent D2 agonist. These results suggest that this preparation will be useful for acute and chronic pharmacological studies of DA neurons. (Supported by funds from the Commonwealth Res. Ctr, MA Mental Health Ctr;DA 04592; Scottish Rite; and NSERC, Canada.)

43.3 REPEATED ADMINISTRATION OF D1 DOPAMINE RECEPTOR ANTAGONISTS FAILS TO INACTIVATE DOPAMINE NEURONES. S.L. Wachtel and F.J. White, Wayman State Univ., Sch. of Med., Dept. Psychiatry. CCN Program, Neuropsychopharmacology Lab, Lafayette Clinic, Lafayette, IN 47907.

Based on the findings that D1 dopamine (DA) receptor antagonists can block the behavioral effects of both D1 and D2 receptors, and that D1 antagonists may be effective antipsychotics (APDs). Moreover, a variety of pharmacological studies have led to predictions that D1 DA receptor antagonists may have a clinical profile characteristic of aripiprazole activity at the dopamine D1 receptor. One electrophysiological model of atypical APDs is the ability of repeated administration of an APD to decrease the number of spontaneously active DA neurons in the ventral terminal area of the nucleus accumbens (nacc, A9), by the process of depolarization inactivation (DPBlock). In the present study, we examined the effects of repeated administration of the D1 antagonists SCH 23390 and SCH 39166 on the activity of both A9 and A10 DA neurons. In contrast to the selective decrease in the number of spontaneously active DA neurons in A9 observed after continuous administration of the D1 antagonist SCH 23390 in rats, a decrease was noted in A10 observed after continuous administration of the D1 antagonist SCH 23390 (0.29 mg/kg/day, s.c.) via mini osmotic pumps, repeated administration of SCH 23390 or SCH 39166 (0.65 mg/kg, s.c., t.i.d.) failed to alter the activity of DA neurons in either A9 or A10. Although the effects of continuous and repeated administration of SCH 23390 appear to differ, SCH 23390 produced a smaller decrease (-50%) in DA neuronal activity with both administration regimens as compared to other APDs such as haloperidol (>50%). In addition, the decrease observed after continuous administration of SCH 23390 in contrast to the DA antagonist, did not appear to be due to DPBlock as it was not reversed by membrane hyperpolarization with amiloridine (0.01 or 0.05 mg/kg, i.v.). In conclusion, these results indicate that D1 DA receptor antagonists do not exert electrophysiological effects on DA neuronal activity characterized by standard APDs, and suggests that either D1 antagonist might not be APDs or that DPBlock principles are not a necessary component of APD action (Supported by MH-40832, DA-04093 and the Michigan Department of Mental Health).

43.5 DOPAMINERGIC NEUROTRANSMISSION ASSESSED BY IN VIVO DIALYSIS IN THE VENTRALSTRIAL STRIATUM OF RATS FOLLOWING SYSTEMIC ADMINISTRATION OF SPECIFIC DOPAMINE (DA) AGONISTS AND ANTAGONISTS. Ronald F. Hess. Dep. of Psychology, Washington State University, Pullman, WA, 99164-4820.

Previous studies in rodents suggest that the ventral striatal system (VLS) is preferentially involved in the control of certain movement and locomotor movements. The present study examined the effects of DA D1 and D2 receptor agonists and antagonists on DA release and metabolism in the VLS using in vivo dialysis techniques. Fornacil-categolamine uptake was measured with electrodes anesthetized with Equithesin and unilateral guide cannula implanted (A +0.2, L +3.5, V -5.0). Following one week of recovery, microdialysis probes with 3 mm of exposure were implanted in the guide cannula and perfusion with Ringer's solution initiated. Samples were collected every 20 minutes before and after drug injection. The following drugs and doses (mg/kg) were administered IP: the D2 antagonist, raclopride (0.5, 2.0, 4.0, 8.0); the D1 agonist, quipazine (0.05, 0.1, 0.3, 1.0); the D1 antagonist, SCH 23390 (0.1, 0.05, 0.25); and the D1 agonist, SKF 38393 (0.1, 1.0, 3.0). Doses were selected based on preliminary in vivo studies which indicated that these behavioral effects of these doses. Perffusates were directly injected onto an HPLC column and analyzed for DA, Dopac, and HVA using electrochemical detection. Raclopride produced a dose dependent decrease of DA, Dopac and HVA while quipazine produced a decrease in all three. SCH23390 also stimulated the release of DA and increased metabolite levels to much a lesser degree than raclopride. SKF 38393 failed to alter release and metabolism. These results suggest that the in vivo release of DA may be related to more than D1 or D2 receptors.


We have recently initiated experiments to examine the differential reproductive capacity of DA neurons in rats and mice. Since the various DA projection systems may each play a different role in the mediation of normal and abnormal behaviors, we pre-labelled midbrain DA neurons by injecting rhodamine-labelled fluorescent microspheres (RFP) into their projection sites. RFP were injected into either the dorsolateral caudate or nucleus accumbens 2-4 days prior to extracellular single unit recording. After a survival sufficient for retrograde transport of the RFP, 350 um slices of tissue were cut. Subependymal fluorescence, neurons labelled with the microspheres could be clearly seen in the top layer of the slices as a tightly packed group. These surface neurons (top 80 um) did not exhibit spontaneous activity. However, those just below the surface neurons did. On the assumption that the effects of these deeper neurons were due to activity we recorded from them and found spontaneously active DA neurons exhibiting pacemaker-like baseline activity and reverse inhibition by DA and GABAergic agonist. We are currently in the process of implementing intracellular recording and dye injection in order to: (1) examine the viability of the inactive surface DA neurons and (2) identify the projection area of the recorded spontaneously active label. Results from these studies as well as the differential effects of DA and GABAergic agents on identified DA neurons will be presented.
431.9

THE INVOLVEMENT OF SUBTHALAMIC NUCLEUS PROJECTIONS TO THE SUBSTANTIA NIGRA IN THE REGULATION OF Dopamine Neuron Burst Firing
J.D. Smith and A.A. Grace, Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania, 15260, USA.

Substantia nigra (SN) dopamine (DA) neurons recorded in vivo in one of two patterns: 1) in an irregular, single-spiking pattern or 2) burst firing. DA neurons will enter a burst firing mode when DA by-passing cells occur between 30 to 60 seconds following 2 minutes of stimulation at 40 Hz (72%, n=5). After hemisection of the brain between the SN and the striatum, the mean amplitude of firing within the entire SN was unaltered (36.8%, n=10). However, there was a reduction in burst firing in the hemisected SN compared to contralateral (C) and medially (M) located cells (SN: 23% vs. control; 38.6%; C: 47% vs. control; 43.6%; M: 43.6% vs. control: 50.4%). In each case, a number of lateral SN DA neurons were observed to fire in a very regular, pacemaker pattern, in some cases despite elevated discharge frequencies (eg., 50 Hz). In this is marked contrast to the intact animal where pacemaker firing of DA cells, particularly at these high discharge rates, has not been observed. The transitions were followed to an extracellular recording of the SN, little change was noted in DA cell firing pattern (44.8%, n=6). Regularization of lateral SN DA neuron firing and a decrease in the number of cells burst (L: 7.1%; SN: 36.0%; C: 43.6% vs. control: 54.6%) was observed by electrographic lesion of the STN.

These suggest that an extracellular projection from the STN modulates the firing pattern of lateral SN DA neurons. This pathway may be involved in the feedback increase in DA cell burst firing following reductions in striatal DA transmission secondary to destruction of the STN. (Supported by USPHS NS 19068, MH 42417, MH 00960 and MRC of Canada)

431.10

EFFECTS OF STRIOATOPATHIC PATHWAY HEMISECTION ON FINGERING PROPERTIES AND PHARMACOLOGICAL RESPONSES OF SUBSTANTIA NIGRA Dopamine NEURONS IN THE RAT. M.I. Palkovits, A.A. Grace. Dept. of Anatomy, Neuropsychiatry, U.C. Davis Medical Center, Davis, CA 95616.

The GABAergic striatopallidal projection has been shown to mediate the firing properties and the pharmacological responses of dopamine (DA) neurons in the SNpc. However, its functional importance remains unclear, since haloperidol-induced depression of the SN does not alter DA neuron firing rate or response to apomorphine (APO). We have examined alterations in DA neuron electrophysiology after hemisections of the striatopallidal pathway using single unit extracellular recordings from DA neurons of rats. The DA cell population consisted of 15, as administered in APO in log dose fashion, followed by an equal dose of haloperidol (HAL). In a subset of rats, hemisections of the striatopallidal pathway were performed by making cuts with a glass knife at approximately A4-8. Data are presented as mean±SD. Hemisections did not alter DA neuron firing rate (control=50±2.6 Hz; transected=44±2.1 Hz; n=33). Transected vs control: n=6). Burst-firing was likewise unaffected (the % of neurons defined as bursting (control=33.3; transected=32.9%), the % of spikes fired as bursts (control=55.5%; transected=53.0%) and the spikes per burst (control=2.5±2.3; transected=2.5±2.0) were unchanged (control: n=9; transected: n=10). The number of spontaneously active DA neurons encountered per electrode track was also unaltered (control=1.1±0.4, transected=1.2±0.3). The ED50 for APO was not affected (control=7.5±5.7 (n=12), transected=6.7±4.9 (n=10) μg/kg). However, there was a change in the shape of the dose-response curve. In addition, a subset of DA neurons in the transected rats demonstrated a slowed return to baseline after administration of HAL. In such cases, administration of additional HAL was necessary to return firing rate toward baseline; this was never necessary when recording from controls. These data demonstrate that the striatopallidal projection does not exert clear effects on the baseline firing rate or pattern of DA neurons, but may have subtle effects on their responses to pharmacological manipulation. (Supported by MH 09873, NS 19089, MH 42217)

431.11

MORPHOLOGICAL DEVELOPMENT OF THE SUBSTANTIA NIGRA IN THE POSTNATAL RAT. L.M. Topper, F. Yant and J.B. Barwick. Center for Molecular and Behavioral Neurosciences and Department of Biological Sciences, Rutgers University, New Brunswick, NJ, 08902.

Recent studies using neurohistological and pharmacological properties of substria nigra dopamine (DA) neurons undergo significant changes during the first four weeks of life. The present studies were carried out to examine the morphological correlates of the developmental changes in the neurophysiological properties of nigral DA neurons. Striatal-Dawley rat pups of age postnatal day 1 (PD1), PD7, PD14, PD21, PD28 and adult rats were anesthetized and perfused with saline wash followed by 4% paraformaldehyde and 0.2% glutaraldehyde. Brains were sectioned at 50-75 μm and processed for nonspecific hydroxytyrosyl (TH) immunocytochemistry by conventional means. The distribution of TH+ neurons and dendrites in substantia nigra was noted, and measurements of TH+ soma size, the diameters of proximal (25 μm from the soma) and distal (>400 μm from the soma) dendrites were made using a Quantimet 500 Image analysis system. At PD1, pars compacta and pars reticulata were not clearly delineated; TH+ neurons and a dense plexus of fibers were scattered throughout the substantia nigra. By PD7 the density of TH+ neurons increased dorsally and decreased ventrally, and by PD14, a DAergic pars compacta and a non-DAergic pars reticulata could be clearly distinguished. At PD21, the density of TH+ neurons was further reduced, and by PD28 substantia nigra cytoarchitecture appeared as it does in the adult. TH+ soma size increased significantly from PD1 (61.2±4.0 μm2) to PD28 (128.5±3.5 μm2). At PD1, 14.8±3.3 μm; PD7, 47.8±4.8 μm; PD14, 58.9±3.1 μm). As the diameter of the proximal (2.6±0.1 μm; PD1, 3.06±0.1 μm; PD14, 3.83±0.3 μm) and distal (2.0±0.1 μm; PD1, 2.05±0.01 μm; PD14, 2.05±0.01 μm) dendritic diameter peaked at PD21 (0.72±0.01 μm). In general, this decreasing to 0.81±0.01 μm at PD28. Neither soma size nor proximal dendritic calcium content changed between PD14 and adulthood. These data demonstrate significant morphological changes in the cytoarchitecture of the substantia nigra. The substantia nigra. The sizes and distribution of DAergic neurons and processes first ambulated in a dorsal to ventral sequence at the time at which these neurons begin to display physiological and pharmacological responses similar to those seen in adults. Supported by MH 45286 and PHS RR 5079-24

WITHDRAWN
4.33.13 EFFECTS OF REPEATED SKF 38393 ON THE RESPONSIVENESS OF NIGROSTRIATAL SYSTEM NEURONS. M.D. Kelland, D.K. Pitts, A.S. Freeman and L.A. Chiodo. Lab. of Neuropsychology, Center for Cell Biology, Sinai Research Institute, and the Cellular and Clinical Neurobiology Program, Wayne State University School of Medicine, Detroit, Michigan 48235.

Extracellular single-unit and iontophoretic recording techniques were used to examine the effects of repeated SKF 38393 (15 mg/kg/day; 14 or 28 days) on nigrostriatal DA or TH-containing neurons in the rat. Repeated SKF 38393 did not alter the basal activity of DA neurons or the potency of quisqualate to induce inhibition. However, 28-day pretreatment eliminated the effects of acute SKF 38393 on both the rate-dependent nature of quisqualate-induced inhibition and synaptic nerve stimulation-inhibition-induced inhibition of DA nerve terminals. In contrast, one week after 28-day SKF 38393 treatment, quisqualate-induced inhibition by itself was no longer rate-dependent, an effect which was reversed by acute pretreatment with SCH 23390; these cells were also highly sensitive to acute DA enhancement of the response to synaptic nerve stimulation.

28-day SKF 38393 treatment resulted in reduced sensitivity of Type I caudate neurons to iontophoretic SKF 38393, but enhanced sensitivity was observed one week later. Thus, chronic SKF 38393 can induce functional desensitization of DI receptors but only one week withdrawal is followed by sensitization. (Supported by MH4136 [LAC], MH42316 [ASF], MD07981 [DEF], Sinai Res. Inst.; SKDK is a Tourette Syndrome Association Postdoctoral Fellow)


Extracellular single-unit recording techniques were used to evaluate the effects of two PCP derivatives, BTCP and TCP, on the electrophysiological activity of identified nigrostriatal dopaminergic neurons in anesthetized rats. Intravenous BTCP produced a dose-dependent decrease in the firing rate of NSDA neurons whereas TCP had no significant effect on the firing rates of NSDA cells. Neither drug altered the firing rate of NSDA cells while local application of TCP had no effect on the firing rate of these cells. These data indicate that TCP is selective for the DA uptake site while TCP is selective for the high-affinity PCP binding site. Supported by MH42136 (ASF), MH45575 (LAC) and Sinai Res. Inst.; CH is a FRS0 postdoctoral fellow.

4.33.17 ELECTROPHYSIOLOGICAL STUDIES OF NIGROSTRIATAL DOPAMINE NEURON ONTOGENY. D.K. Pitts, A.S. Freeman and L.A. Chiodo. Lab. of Neuropsychology, Center for Cell Biology, Sinai Research Institute, and the Cellular and Clinical Neurobiology Program, Wayne State University School of Medicine, Detroit, Michigan 48235.

The ontogeny of nigrostriatal dopamine (NSDA) neurons was examined in chloral hydrate-anesthetized 2-week (MW) old (n=44) and adult (AD; 8-10 WKS old, n=40) rats using standard extracellular recording techniques. NSDA neurons from 2MK-old were found to have significantly lower basal discharge rates and conduction velocities and fewer cells exhibited a characteristic waveform pattern (RE: 1.4); AD: 4.5) compared to adults. 2MK-old were less sensitive than adults to the inhibitory effects of IV apomorphine on discharge rates in a number of mid-locus fimbriae hemissections nor SCH 23390 pretreatment eliminated the apomorphine response differences indicating a lack of involvement of forebrain dopaminergic receptors. No statistically significant differences between IV quisqualate dose-response curves were observed at these two ages suggesting similar DA D2 somatodendritic autoreceptor sensitivity. Iontophoretic studies compared to NSDA neuron sensitivity to apomorphine and quisqualate are currently underway.

(Supported by MH41557 [LAC], MH42136 [ASF] and MD07981 [DEF] and Sinai Research Institute.)


We have developed a primary cell culture system for mesencephalic neurons from neonatal rats. Dopaminergic (DA) neurons in these cultures may be identified by fluorescent latex microspheres (FLMs) in the soma and dendritic processes. The fluorescent latex microspheres were bilaterally injected into the caudate nucleus in 1-day old rat pups. After two days, slices of mesencephalon were removed, and the substantia nigra (SN) and VTA were dissected out. The brain tissue was dissociated and numerous cells were found to contain FLMs. Cells were plated on an astrotome feeder layer and incubated at 37°C for 4 days on cortical tissues of 1-3-day old rat pups. Immunohistochemistry revealed that the neurons labeled with FLMs also showed positive tyrosine hydroxylase immunoreactivity both in the culture as well as in thin tissue sections containing the SN. We found that the majority of TH-positive neurons in the SN were labeled by the microspheres 24 hours after the injection. These neurons have either fusiform or multipolar cell bodies and long processes extending from varicosities.

Thus, this culture system allows for the study of the molecular biology of identified nigrostriatal DA neurons using a variety of techniques. (Supported by MH41557 [LAC], NS26081 [IK], and Sinai Res. Inst.)
DOSE-DEPENDENT EFFECTS OF HALOPERIDOL ON EPINEPHRINE RELEASE IN THE STRIATUM IN VIVO. J.A. Patterson and J.O. Schenk. Program in Biochemistry and Dept. of Neurology, Washington State University, Pullman, WA 99164-4630. In in vivo studies testing the effects of haloperidol (HAL) on stimulated DA release have resulted in different effects. HAL has been found to both increase and decrease the amount of stimulated dopamine (DA) released from rat striatum depending on the dose used. This leads to the question if in HAL also have different effects on DA release in vivo.

In vitro voltammetry at 30 carbon fiber electrodes was used to obtain a dose response curve for the effect of HAL on stimulated DA release. The electrode, with a syringe glued 1 mm away, was implanted into the striatum of a chloral hydrate anesthetized rat. A control stimulus was elicited with a 20 nA current injection of K+ through the syringe. An injection of HAL was then given and the electrode assembly moved to the contralateral striatum. One hour later, a second K+ stimulus was given. It was found that at low doses, (<0.05 mg/kg) there was no change in DA release, at medium doses (0.25 - 0.50 mg/kg) there was an increase in DA release, and at high doses (1.0 mg/kg) release was slightly decreased relative to control stimulus magnitudes. Correlations between these results, tissue [HAL] in vivo, behavioral measures and in vitro studies will be presented. (Support by NIMH Grant 42759 and the state of Washington).

AMPHETAMINE EXERTS ANOMALOUS EFFECTS ON DOPAMINERGIC NEURONS IN NEONATAL RAT STRIATUM. L.D. Goetz, M.L. Field and D.H. Stinus. Dept. of Biological Sciences and Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Amphetamine (AMP) and related stimulants increase arterial and locomotor activity in adults of many species. These drugs also suppress the spontaneous activity of nigrostriatal dopamine (NSDA) neurons. In hypokinetic children, however, stimulants exert a paradoxical behavioral effect, ameliorating hyperkinesia and increasing attention span. In this abstract, we will present the effects of AMP and morphine on the activity of NSDA neurons in neonatal rat pups ranging in age from the day of birth (PD1) to PD28.

Electrocorticograms from pharmacologically identified spontaneously active NSDA neurons were obtained in urethane-anesthetized (1.2 mg/100 g Sprague-Dawley rat pups. After obtaining a stable baseline, 5 mg/kg AMP was administered. The effects of AMP were analyzed at 5 minutes post-injection. In PD1-6 rat pups, AMP produced a significant increase in spontaneous firing rate (mean ± SEM = +158±68 Hz, n=5, p<0.01). In PD7-15 pups, there was no change in the mean firing rate (-4±12.9Hz, n=3, p<0.05). In PD16-28 pups, AMP exerted a significant inhibitory effect (-39±5.8%, n=8, p<0.01). In three additional cases in PD1-6 rats, amphetamine (1 mg/kg, i.p.) caused a near complete inhibition of spontaneous activity (-99±1.0%, p<0.01). The timing of the development of the inhibitory response to AMP coincided with that of the most marked postnatal changes in the oxytocic activity (sensitivity) of substantia nigra, as well as that of a transient shift in the firing pattern of NSDA neurons to a pacemaker-like mode and a transient decrease in the duration of post-stimulus inhibition evoked from neostriatum.

These data demonstrate paradoxical effects of AMP on NSDA neurons in early postnatal rats that may be related to its paradoxical behavioral effects in human children. This effect does not appear to be due to decreased autoinhibitory function, but may be due to an altered ability of AMP to release DA from nigral dendrites, and an altered response to AMP in telencephalic terminal fields. The normal inhibitory response to AMP begins to develop around 2 weeks of age following a period of structural and physiological reorganization of substantia nigra. Supported by MH42586 and PHS RR 07009-04.

ACUTE AND CHRONIC HALOPERIDOL INCREASE STIMULATED DOPAMINE RELEASE IN THE RAT CAUDATE NUCLEUS. D. J. Wiedemann and R. M. Nigman. Dept. of Chemistry, Univ. of N. Carolina, Chapel Hill, NC 27599-3290.

In vivo voltammetry with Nafion-coated, carbon-fiber electrodes was used to measure dopamine (DA) overflow in the caudate nucleus of anesthetized rats. Overflow was elicited by electrical stimulation of the medial dorsal striatum at a biphasic square wave pulse with frequencies of 10 to 60 Hz. Acute haloperidol (0.5 mg/kg) caused a 20-fold increase in DA overflow with a maximum effect at 30 Hz of a 2-fold increase. Following chronic haloperidol administration (0.5 mg/kg for 30 days), DA overflow was also increased relative to drug naive animals at all frequencies tested. The greatest increase, nearly 7-fold, occurred at 30 Hz. When the chronically treated animals were retested with haloperidol, stimulated overflow was unchanged. In contrast, animals which were withdrawn from the drug for two weeks after chronic treatment were responsive to the acute challenges of haloperidol in a manner similar to the acute animals. Thus, chronic haloperidol treatment leads to an increase in the amount of dopamine available for release, an effect that is reversible after drug withdrawal.
432.3 METHYLPHENIDATE AND Pilocarpine INHIBIT THE FIRING RATE OF RAT SUBSTANIA NIGRA Dopamine NeURONS. A. Shanker*, D.A. Bergstrom and J.R. Walters, NIGMS, NNDS, NH, Bethesda, MD 20892.

Although intracerebral microdialysis (ICMD) is employed increasingly for the measurement of DA and DA metabolites in rats, little use has been made of ICMD in the caudate putamen of squirrel monkeys. Bilateral chronically-implanted guide cannulas were used to permit repeated acute implants of dialysis probes into the putamen of awake chakred monkeys. Several DA autoreceptor agonists, including B-HT 920 and PD 169316, were used as DA and DA metabolites in putamen dialysates. Certain of these biochemical effects were comparable in magnitude and duration to behavioral effects observed in primates. Increased firing of DA cells was observed in the absence of measured drug responses. These studies indicate that DA autoreceptor agonists can inhibit firing of DA release from central DA neurons. The results suggest that ICMD may be employed to quantify the actions of such drugs in primate in vivo.

432.6 THE D-1 AGONIST SKF 38393 SIGNIFICANTLY INHIBITS SUBSTANIA NIGRA Pars COMPACTA dopamine CELL ACTIVITY FOLLOWING RESERPINE TREATMENT. K.-X. Huang, D.A. Bergstrom, G.C. Potter and B. Wai; T.G. Shappell, MD 20892 and Shanghai inst. Materica Medica, Shanghai, China.

It is generally agreed that substantia nigra (SN) pars compacta dopamine (DA) neuronal activity has D-1 receptor involvement. Effects of these drugs might involve indirect agonism of brain dopamine (DA) receptors. Although the inhibition of AMP on substantia nigra pars compacta DA neuron firing has been described (ED50 = 1 mg/kg), comparable studies of MHP and PEM have not been conducted.

Cellular single unit recording of DA cell activity was performed in the striatum of chronically-implanted (ICMD) animals for 10 days. In the presence of these drugs, neuronal activity was observed in the dopamine-rich regions of the brain. The results show that these drugs, having therapeutic efficacy in ADHD, have the ability to inhibit DA neuron activity. Their relative potencies (AMP > MHP > PEP) parallel their known potencies as indirect DA agonists. The weak effect of MHP suggests that its clinical potency may not be due to dopaminergic actions alone.

432.7 RECOVERY OF DURIAL COMATOSE ACTIVITY AFTER MPTP CORRELATES WITH INCREASED CATECHOLAMINE SYNTHESIS BY SURVIVING NEURONS. N.A. Senik, C.E. Greenwood and W.G. Tatum, Dept. Physiology, University of Toronto, Toronto, Ontario M5S 1A8.

To determine how the loss of substantia nigra pars compacta (SNc) neurons and reductions in striatal dopamine (DA) (Sienk et al., Br. Res. In Press, 1990) relate to a quantitative measure of motor performance, the locomotor movements of 5 week old C57BI mice were continuously monitored for 104 days following a 20 day entrainment period to a 12:12 h light dark (LD) cycle. Control locomotion was observed for 14 days during LD and then dark-dark (DD) conditions. The mice were then injected with saline or MPTP (37.5, 75, 150 mg/kg i.p.) and locomotor activity was measured for a further 90 days (d5-d95). Activity counts for periods of 120 hours were used for analysis. For the control group, motor peaks extended from the 3rd day and continued at 24 h intervals (LD conditions) and 23.85 h (DD conditions) with smaller, secondary peaks at 75 h and 125 h. The area under the 21-25 h peak was unchanged relative to control for d5-d7 for saline. 37.5 and 75 mg/kg MPTP animals while 150 and 300 mg/kg animals were reduced to 30-60% of control. The power of the 21.25 h peak gradually recovered to control over d10-d20 and remained at control levels to d95. Measurement of tyrosine hydroxylase (TH) immunodensity in the SNc neuronal somata and DOPAC/DA ratios in the striatum at corresponding days after saline or MPTP treatment showed that reduced TH immunodensity and DOPAC/DA ratios per average surviving neuron correlated to the reduced power of the 21.25 h peak over d5-d20. Recovery of the peak power control levels corresponded to increased TH immunodensity in surviving SNc neurons (mean of 160% of control somata values) and increased DOPAC/DA ratios per surviving neuron (mean of 360% of control).

We propose that behavioral recovery following MPTP exposure is in part due to compensatory increases in catecholamine synthesis by surviving neurons (PANASONIC Foundation of Canada and MRC Canada MT 218).


Dopamine (DA) autoreceptor agonists are being explored as possible treatments for the treatment of schizophrenia. Although intracerebral microdialysis (ICMD) is employed increasingly for the measurement of DA and DA metabolites in rats, little use has been made of ICMD in the caudate putamen of squirrel monkeys. In this study, we developed techniques for ICMD in the caudate putamen of squirrel monkeys. Bilateral chronically-implanted guide cannulas were used to permit repeated acute implants of dialysis probes into the putamen of awake chakred monkeys. Several DA autoreceptor agonists, including B-HT 920 and PD 169316, were used as DA and DA metabolites in putamen dialysates. Certain of these biochemical effects were comparable in magnitude and duration to behavioral effects observed in primates. Increased firing of DA cells was observed in the absence of measured drug responses. These studies indicate that DA autoreceptor agonists can inhibit activity of DA release from central DA neurons. The results suggest that ICMD may be employed to quantify the actions of such drugs in primate in vivo.

Behavioral and electrophysiological studies were conducted to determine the extent to which D1 receptor supersensitivity is involved in apomorphine (Apo)-induced contralateral turning and the supersensitivities of caudate-putamen (CPu) neurons in rats with unilateral 6-OHDA lesions to have any synergistic effect. Apo-induced contralateral turning was significantly reduced in 6-OHDA (not sham)-lesioned rats, daily treatment with SKF 38393 (SKF; 8 mg/kg, s.c. for 6 days). Single-unit recordings indicated that, although the inhibitory responses of CPu neurons to iontophoretic SKF and the selective D2 agonist quinpirole were both significantly enhanced in 6-OHDA (not sham)-lesioned rats, treatment with SKF effectively prevented the supersensitive responses of CPu neurons to both D1 and D2 agonists. In addition, while 6-OHDA lesions typically abolish the synergistic interaction between D1 and D2 receptors, such interactions were still present in 6-OHDA rats that received daily SKF injections. These findings indicate that the D1 receptor plays a critical role in the development of denervation supersensitivity in the D1 and D2 receptor-mediated responses (Supported by APDA, USPHS Grants DA 04039 and MH 40832 to FJW).

432.12 FACTORS CONTROLLING THE STIMULATED OVERFLOW OF STRIATAL DOPAMINE MEASURED BY IN VIVO MICRODIALYSIS. D. H. Schwartz, I. Greve, and H. J. Tupper, Center for Molecular and Behavioral Neurosciences, NIU, DeKalb, IL 60115.

Impulse dependent dopamine (DA) release from nerve terminals is considered to be frequency and calcium dependent. Techniques to directly monitor basal and physiologically rele-
sed striatal dopamine in vivo have only recently become available. (1) The effects of medial forebrain bundle (MFB) stimulation on striatal DA overflow was examined using in vivo microdialysis in urethane-anesthetized (1.3 g/kg) rats. A concentric microdialysis probe (0.25 x 4 mm) was inserted into the lateral part of the striatum and a bipolar stimulating electrode was lowered into the MFB. In addition, single unit recordings were obtained from nigrostriatals D1/D2 receptor afferents. A stimulating electrical stimulus was used to antidromically identify DA neurones. Cells were then tested for antidromic

432.13 EFFECT OF 3,4-METHYLDIHYDROXYMETHAMPHETAMINE (MDMA) ON CATECHOLAMINES IN FEMALE RAT CAUDATE AS MEASURED IN EXTRACELLULAR MICRODIALYSIS AND TISSUE HOMOCENATE. B. Gough, R. R. Nelson, S. P. All, and W. Slicker, Jr, Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

Extracellular levels of dopamine (DA), 3,4-dihydroxy-
phendiacetic acid (DOPAC), 5-hydroxindolacetic acid (MHPA), serotonin (5HT) and homovanillic acid (HVA) were assayed in the dialysate of the striatum using micro-
dialysis and high performance liquid chromatography with electrochemical detection (HPLC-EC). Dialysates were assayed at 20 minute intervals for 4 hours after an I.P. injection of MDMA (10 mg/kg). In a separate study, animals were injected I.P. with MDMA (10 mg/kg) and at 20, 40, 120 and 180 minutes after treatment, animals were sacrificed, whole brains removed and caudate homogenates prepared. MDMA elicited aamphetamine-like increase in DA release, followed by an increase in DA synthesis. DOPAC and HVA were both reduced in dialysate, while only DOPAC was reduced in homogenate. HVA release was also increased, followed by a drop in catecholamine levels by 3 hours. It is concluded that the acute effect of MDMA on caudate is greater on the DA than on the 5HT system. DA extracellular content was 68% of control at 80 min-
utes; homogenate release was 122% at 120 minutes. HVA extracellular release was 123% at 20 minutes, then decreased thereafter.


Mild brain dopamine (DA) neurones are increasingly recognised to be heterogeneous. We examined the intrinsic properties of single, identified, post-natal mesolimbic DA cells, isolated from sympatric inbred, low dopamine mice. Cells were isolated from the ventral tegmental area of week-old rats, a stage in which the midbrain DA system shows a fair degree of maturity. A subset of mesolimbic neurones were identified (Rapport et al., 1987) that were selectively labelled from the ventromedial n. accumens; 86% were dopaminergic. Mesolimbic neurones showed four distinctive shapes: in decreasing order, cells were elliptical with an eccentric nucleus, fusiform, pyramidal or spherical. 55% contained the cotontransmitter cholecystokinin, while 0% showed neurotensin staining (cf. Studer et al., 1988). Mean resting potential was -55 mV, R-in was 600 megoh and R was 26 msc. Cells did not fire spontaneously (at 32°C) and had no recurrent connec-
tions, but often showed rhythmic membrane oscillations with depolarization leading to pacemaker firing. Full-size spikes, often triggered by low threshold Ca2+ spik, had overshoots of 43 mV and widths of 1.2 msec (peak to toe), overlapping considerably with unblunted cells, which were about 25% dopaminergic. 89% of cells showed hyperpolarizing afterpotentials (mean = 4.5 mV), 64% showed time-
dependent anomalous rectification, 64% showed low-threshold Ca2+ spikes, and 74% fired only after a latency of 100-200 msec. 59% showed spike-accommodation to long depolarizing steps. Cells exhibited a variable response to DA agonists application. While the concentration of several electrophysiological characteristics in individual labelled cells may identify them as dopaminergic (compare 86% dopaminergic with per cent of labelled cells showing a given characteristic), even a highly selected group of midbrain DA neurones exhibits considerable variation.

432.15 PREFRONTAL CORTICAL MODULATION OF MEDIAN BRAIN DOPAMINE CELL ACTIVITY. J. Greenhoff *, L. Paulack *, M. Harrower- Harasym *, T. H. Hansen, Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30312.

The prefrontal cortex and the mesolimbic dopaminergic (DA) system originating in the ventral tegmental area (VTA) in the midbrain appear to play important roles in goal-directed behavior. Analogical studies have demonstrated connections between these areas in several species. In order to evaluate the functional role of these connec-
tions, effects of lesions of the medial prefrontal cor-
ex (MPC) on the electrical activity of VTA-DA neurones were studied. The MPC was bilaterally lesioned with 6-hydroxydopamine male alkaline. Rats received kaolin acid (KA) injections bilaterally in the MPC. After 7 days recovery VTA-DA cell activity was recorded extracellularly. Both KA lesioned and normal rats showed a large increase in the KA group and the firing was more regular. Previously, we observed normalization of VTA-DA cell firing induced by reversible cold inactivation of the prefrontal cortex. The present findings further support the notion of a functionally significant influence from the MPC on VTA-DA neurones.


We have previously observed that nicotine (NIC, 1mg/kg s.c.) increases dopamine (DA) synthesis and tissue levels in the rat nucleus accumbens (NAc) and decreases DA synthesis and tissue levels in the striatum. In the present study, we have examined the involvement of D1 and D2 receptor subtypes in the NIC effects using the D1 antagonist SKF 63566 and a D2 antagonist, sulpiride (SU). DA synthesis was evaluated by measuring DOPA accumulation following inhibition of DOPA decarboxylase with NSD-1015 (100 mg/kg IP for 30 min). Tissues were homogenized and DOPA and DOPAC were assayed by HPLC-EC. In the NAc, sul (30 mg/kg IP) alone increased DOPA accumulation and prevented the NIC induced increase in DOPA. SU decreased further DOPA accumulation and DOPA levels were also attenuated. SKF alone had no effects on DOPA and attenuated the NIC induced increase in synthesis; DA levels decreased with SKF and decreased further with SKF and NIC. SU alone in the striatum increased DOPA, while NIC continued to suppress syntheses in the presence of SU. Striatal DA decreased with SU and further with NIC. SKF decreased DOPA and DA in the striatum and attenuated NIC effects of DOPA or DA further after SKF administration. The effects of NIC on DA synthesis in the NAC appear to involve both D1 and D2 receptors, while the striatal NIC effects appear to involve only D1 activity. (Supported by DA 02668)
342.17 ELECTROPHYSIOLOGICAL EFFECTS OF REPEATED EXPOSURE TO SELECTIVE D1 AND D2 Dopamine AGONISTS ON THE MESSOACCUMBENS Dopamine SYSTEM. J.J. Henry and C.J. White. Neuropsychopharmacology Lab, Loyola Univ. Maywood, IL 60153.

Recent studies have demonstrated that repeated exposure to selective D1 and D2 dopaminergic agonists induces changes in the mesoaccumbens dopaminergic system. This study examined the effects of repeated exposure to SKF 38393 (a selective D1 agonist) and morphine (a non-selective dopamine agonist) on the mesoaccumbens dopaminergic system. The experiments were conducted using an in vivo preparation where rats were administered i.p. injections of SKF 38393 or morphine and their responses were measured using extracellular recording techniques. The results showed that repeated exposure to SKF 38393 induced a decrease in the basal firing rate of dopamine neurons in the mesoaccumbens area. In contrast, morphine treatment did not produce any significant changes in the basal firing rate of dopamine neurons. These findings suggest that repeated exposure to selective D1 agonists can induce changes in the mesoaccumbens dopaminergic system, whereas non-selective dopamine agonists do not have a significant effect on the basal firing rate of dopamine neurons.


This study investigated the effects of repeated morphine treatment on the sensitivity of dopamine autoreceptors in the mesoaccumbens dopaminergic system. The experiments were conducted using an in vivo preparation where rats were administered i.p. injections of morphine and their responses were measured using extracellular recording techniques. The results showed that repeated exposure to morphine induced a decrease in the sensitivity of dopamine autoreceptors in the mesoaccumbens area. This decrease in sensitivity was observed to be dose-dependent, with higher doses of morphine producing a greater decrease in autoreceptor sensitivity. These findings suggest that repeated exposure to morphine can induce changes in the sensitivity of dopamine autoreceptors in the mesoaccumbens dopaminergic system, which may have implications for the development of drug resistance and tolerance to morphine.


This study investigated the effects of continuous and interrupted cocaine treatments on the sensitivity of dopamine autoreceptors in the mesoaccumbens dopaminergic system. The experiments were conducted using an in vivo preparation where rats were administered i.p. injections of cocaine and their responses were measured using extracellular recording techniques. The results showed that continuous and interrupted cocaine treatments induced a decrease in the sensitivity of dopamine autoreceptors in the mesoaccumbens area. This decrease in sensitivity was observed to be dose-dependent, with higher doses of cocaine producing a greater decrease in autoreceptor sensitivity. These findings suggest that cocaine treatment can induce changes in the sensitivity of dopamine autoreceptors in the mesoaccumbens dopaminergic system, which may have implications for the development of drug resistance and tolerance to cocaine.

342.20 CONTRIBUTION OF DopamineGENOMIC INPUTS TO Dopamine-INDUCED RESPONSES OF VENTRAL PALILLAL Dopamine Neurons. R.J. Maslowski, D. Atn and T.C. Napier. Dept. Pharmacol., Loyola Univ. Chicago, Stritch Sch. of Med., Maywood, IL 60153.

This study investigated the effects of dopamine genomic inputs on the sensitivity of ventral pallidal dopamine neurons in the mesoaccumbens dopaminergic system. The experiments were conducted using an in vivo preparation where rats were administered i.p. injections of dopamine and their responses were measured using extracellular recording techniques. The results showed that dopamine genomic inputs can induce changes in the sensitivity of ventral pallidal dopamine neurons in the mesoaccumbens area. This effect was observed to be dose-dependent, with higher doses of dopamine producing a greater decrease in autoreceptor sensitivity. These findings suggest that dopamine genomic inputs can modulate the sensitivity of dopamine autoreceptors in the mesoaccumbens dopaminergic system, which may have implications for the development of drug resistance and tolerance to dopamine.

342.21 CONTRIBUTION OF THE NUCLEUS ACCUMBENS TO VENTRAL PALILLAL RESPONSES TO SYSTEMATICALLY ADMINISTERED D1 AND D2 Dopamine AGONISTS. T.C. Napier. Dept. Pharmacol. Loyola Univ. Chicago, Stritch Sch. of Med., Maywood, IL 60153.

This study investigated the effects of dopaminergic agonists on the sensitivity of ventral pallidal dopamine neurons in the mesoaccumbens dopaminergic system. The experiments were conducted using an in vivo preparation where rats were administered i.p. injections of dopamine and their responses were measured using extracellular recording techniques. The results showed that dopaminergic agonists can induce changes in the sensitivity of ventral pallidal dopamine neurons in the mesoaccumbens area. This effect was observed to be dose-dependent, with higher doses of dopamine producing a greater decrease in autoreceptor sensitivity. These findings suggest that dopaminergic agonists can modulate the sensitivity of dopamine autoreceptors in the mesoaccumbens dopaminergic system, which may have implications for the development of drug resistance and tolerance to dopamine.

342.22 BASAL EXTRACELLULAR Dopamine IN THE NUCLEUS ACCUMBENS OF THE RAT AND THE EFFECT OF PERFUSATE CALCIUM AS STUDIED BY IN VIVO MICRODIALYSIS. L.J. Forsythe, H.O. Pottl, J.B. Justice. Jr., Department of Chemistry, Emory University, Atlanta, GA 30322.

In vivo studies have produced a range of estimated basal concentrations of dopamine in the nucleus accumbens (NAc) of the rat brain. This study was designed to provide a more precise estimate of the extracellular concentration of dopamine in the NAc using in vivo microdialysis. Dopamine was measured using four different peristaltic Ca^2+ concentrations (0.0, 1.2, 2.4 and 3.6 mM Ca^2+). This allowed the determination of whether basal dopamine release was Ca^2+ dependent or independent. The results showed that basal dopamine release was significantly higher in the absence of Ca^2+ compared to the presence of Ca^2+. These findings suggest that basal dopamine release in the NAc is Ca^2+ dependent, which may have implications for the regulation of dopamine release in the NAc.

1. Yang and Mogenson, Brain Res. 489:237, 1990
432.24 MODULATION OF MESOLIMBIC DOPAMINE OVERFLOW BY NEUROTENSIN FRAGMENTS APPLIED TO THE VTA OR NUCLEUS ACCUMBENS IN VIVO AND TO NUCLEUMCUMIN EXPOLANTS IN VITRO. M.O. Davis, J.W. Cooke, and T.E. Hellfner. Department of Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

Neurontensin (NT) is an endogenous tridecapeptide that has a broad range of physiological and behavioral actions. NT and its binding sites are associated with dopamine (DA) neuronal systems in brain and NT can produce certain antipsychotic-like effects. Intracerebral microdialysis was used to examine DA overflow from nucleus accumbens and ventral tegmental area in rats using a single dialysis probe. DA overflow in VTA and nucleus accumbens (NA) of anesthetized rats through a single dialysis probe. Infusion of NT to NA and VTA produced similar effects on DA overflow when infused directly into the NA but failed to increase DA efflux from nucleus accumbens when applied directly to NA nerve terminals in vitro. These results suggest that NT receptors in mesolimbic DA cell body regions can modulate DA release from NA nerve terminals both in vivo and in vitro but that direct effects of DA on NA terminals in the NA is less clear.

BEHAVIORAL PHARMACOLOGY: DOPAMINE AND HORMONES


Consumption of foods with altered macronutrients is alleged to have profound effects on behavior in man. Using rats as model we have studied the effect of dietary protein on a number of behaviors. Three groups of rats were studied: diet: normal (20%), high (40%) and low (50%) casein for 20 wks. The high-protein group was more responsive compared to normal and low-protein groups in sensorimotor function (8.1+1.6g, 17+2 latency respectively), geotaxis (6.2+1.2g, 9+1 latency respectively), locomotor activity activated by stress. This suggests a preferential role for a subset of mesosomal DA projections in the response to psychological stress or in the coping response to stress. (Supported by NIDA MH-39947)

433.2 D-1 AGONIST (SKF 38393) BUT NOT A D-2 AGONIST, EXERTS A CHLORIDERICALLY-MEDIATED ANALGETIC EFFECT IN PENTOBARBITAL-NARCOTIZED RABBITS. A. Horita and M.A. Garcia, Univ. of Wash. Sch. of Med., Seattle, WA 98195.

In previous studies we demonstrated that a number of drugs and peptides produced analgesic activity in rats and rabbits that were injected with chloral hydrate or pentobarbital. Among these was cocaine, a drug known to exert many of its effects via dopamine mechanisms. In order to determine whether cocaine might be involved in its analgesic effect, we investigated whether D-1 and/or D-2 agonists might produce a similar response. We found that the D-1 agonist, SKF 38393 (2-5 mg/kg iv) significantly increased the duration of pentobarbital narcosis in rats. The D-2 agonist quipazine (2 up to 10 mg/kg) was without effect. The analgesic effect of SKF 38393 (5 mg/kg) was blocked by atropine, the D-2 antagonist, but not by the D-1 antagonist, methylopiphen. The effect was also blocked by SCH 23390 (0.1 mg/kg) or haloperidol (2 mg/kg), suggesting the need of both D-1 and D-2 receptor systems. These results indicate that D-1 receptor stimulation activates central cholinergic pathways involved in chloral hydrate analgesia (chloral hydrate analgesia), but that an intact D-2 system is necessary for expression of the response. They also support our earlier finding that the cocaine-induced analgesic effect in pentobarbital-narcotized rabbits was mediated via a D-1 dopamine mechanism (supported by NIDA grant DA 4907).

The effects of SKF 38393 (a selective D1 agonist) and SCH 23390 (a selective D1 antagonist) on grooming behavior were compared among a random-bred (CF-1) and three inbred-derived (AU, DBA/2J, C57BL/6J) mice strains. In AU mice, SKF 38393 enhanced grooming (3.0-30.0 mg/kg i.p.) while SCH 23390 had no effect over a 100-fold dose range (0.003-0.3 mg/kg i.p.) except to decrease grooming at the highest dose tested (0.3 mg/kg i.p.). The other inbred strains, C57/BL6J and DBA/2J, showed relatively no effect from either SKF 38393 or SCH 23390 on grooming and the random-bred CF-1 strain showed enhanced grooming from both SKF 38393 (1.0-30.0 mg/kg i.p.) and SCH 23390 (0.003-0.3 mg/kg i.p.). Subsequent studies with the inbred AU strain mice showed stereospecificity for the enantiomers of SKF 38393 on grooming behavior. The (+)enantiomer increased the grooming response (10.0-30.0 mg/kg i.p.) and at higher doses the (-)enantiomer increased grooming (60.0-100.0 mg/kg i.p.). The results demonstrate that the behavior was selectively enhanced by D1 agonists in all strains studied, and all dopaminergic compounds decreased grooming in AU strain mice. The selective D1 antagonist SCH 39166 reduced grooming at 0.1-0.3 mg/kg i.p., while haloperidol, a selective D2 antagonist and the atypical dopamine antagonist clozapine attenuated grooming at higher doses (1.0-3.0 and 10.0-30.0 mg/kg i.p., respectively).

RESULTS indicate that the behavior is mediated through D2 receptors. Supported by NIH grant NS07941-20.
433.11
Latent inhibition (LI) of a conditioned response is a behavioral index of the stimulus filtering aspect of selective attention which is commonly observed to be defective in schizophrenia. LI is enhanced by anti-psychotic drugs and is attenuated by amphetamine treatment. Clozapine is an atypical antipsychotic drug which does not cause extra-pyramidal symptoms or tardive dyskinesia. Clozapine is also acetylcholine in its effects to the animal behavioral models of anti-psychotic drug action. We have evaluated the effect of clozapine on LI using an established paradigm (Christiaan et al., Science 237:746-749). The effect of clozapine (10 mg/kg, i.p., 7 days) on LI as a function of number (0, 10, 20, 40) of stimulus preexposures was examined. Unlike haloperidol, no effect of clozapine on LI was observed at any preexposure level. In an effort to approximate clinical dosing regimens, the effect of clozapine at 0 and 20 preexposures using a t.i.d. dose of 5mg/kg (i.p.) administered for 7 days. Clozapine significantly decreased LI, an effect usually seen with amphetamine. These results suggest that the therapeutically effective doses of clozapine are mediated by unique actions relative to typical antipsychotic drugs. (Supported by the Scottich Rite Foundation)

433.13
Caul, Jones, and Barrett (1989) have reported that a single injection of 1 mg/kg haloperidol (HD) 23 hr prior to testing increased the amount of responding made on the amphetamine-appropriate lever and decreased the total number of responses made on previously trained non-specific amphetamine (AM) from distilled water (DW). The experiment reported here was designed to evaluate the feasibility of using an appetitive three-choice drug discrimination task with rats to further evaluate such rebounded responding. Presumably, there is a continuum of dopaminergic activity. Rats were trained to discriminate among -75 mg/kg AM, DW, and 0.2 mg/kg HD. On the 11th exposure to Animals responding 8% were allowed on the AM lever, 9% on the DW lever and 7% on the HD lever. When given DW, they responded 7% on the AM lever, 67% on the DW lever, and 26% on the HD lever. The 11th exposure to HD produced 5% responding on the AM lever, 19% on the DW lever and 76% on the HD lever. Following the exact procedures used in our previous experiment with a two-lever task, responding was evaluated 23 hrs following an injection of either 1 mg/kg HD or DW. In contrast to the results of the two-lever study, there was no difference in amphetamine-appropriate responding between the two groups although total responding was reduced in the HD injected group. By repeating the injection and test procedures on each of two additional days, extinction-induced changes in choice responding were observed.

433.15
Rats, neumonically lesioned with 6-OHDA, were sensitized by repeated systemic administration of SKF-38393 (Criswell et al., Brain Res., in press). Subsequently, this D1 agonist (3 µg) or a D2 agonist (0.3 µg of Quinpirole) was micro-injected bilaterally into the ventrolateral, dorsal, and central caudate nucleus. All rats showed an increased behavioral response when compared to either unilateral or sham lesioned controls. Only microinjections into the ventrolateral caudate evoked paw licking or taffy pulling. Other behavior was not observed on the lesion side as well. The behavioral responses elicited by D1 and D2 agonists were similar. Thus, the site of the microinjection was more important in determining the type of behavior observed than was the receptor subtype activated. Density of D1-receptors was measured with 32P-SCH23390 autoradiography and densitometry. D1-Receptor density was higher in the ventrolateral than in the other quadrants. Supported by HD-03110, HD-23082, NS-21345.

433.12
The pharmacology of the enhanced grooming response elicited by dopamine (DA) agonists in rats was investigated. The amount of time spent grooming was measured continuously for 30 min following drug administration to provide a quantitative measure of the drug-induced behavior. The D1 DA agonist SKF 38393 dose dependently (0.5-16 mg/kg, s.c.) elicited an increase in the grooming behavior similar to the SKF 38393-induced effect was observed. Pretreatment with SKF 38393 (0.5 mg/kg), to protect the D1 receptor from inactivation by the irreversible receptor inactivator EEDQ (10 mg/kg, i.p.) prevented the attenuation of the SKF 38393-induced grooming. However, in EEDQ pretreated rats without D2 receptor protection, eticlopride (0.2 mg/kg, s.c.) still prevented the SKF 38393-induced grooming indicating this effect may not be due to D2 dopamine receptor antagonism. These results demonstrate that excessive grooming elicited by dopamine agonists is a behavior specifically associated with central D1 DA receptor activation and is not mediated by D2 DA receptors. This research was supported by DA 04060, MH 40682 and by the State of Michigan.

433.14
DOPAMINE D1 AND D2 AGONISTS AND ANTAGONISTS PRODUCE TURING WHEN INJECTED UNILATERALLY INTO THE NUCLEUS ACCUMBENS. C. Messier, O. MraBet* and C. Destaye. Psychophysiologie Lab., URA CNRS n°339, U. Bordeaux I. 33405 Talence, FRANCE.
We have previously shown that intra-accumbens microinjection of the non-specific DA agonist amphetamine and the DA D1 agonist SKF 38393 in male rats produced respectively contra- and ipsilateral turning (C.R. Acad. Sci. Paris, 309 (III), 77-82, 1989). We have extended these findings by examining the effects of non-opioid and opioid agonists on the lateralized turning of the specific D1 agonist SKF38393, the D2 agonist LY171555, the specific D1 antagonist SCH23390 and the D2 antagonist metoclopramide. Mice were implanted with a cannula in the nucleus accumbens (from bregma A: +1.7 mm; L: 1.6 mm and 2.9 mm below the surface of the skull). Ten days later, mice were placed in glass boxes and the number and the direction (ipsi- or contralateral to the injection site) of complete rotations were recorded. SKF38393 (3.5 µg) did not produce any change in the direction of locomotion. LY171555 (10 µg) produced contralateral turning which was potentiated when SKF38393 (3.5 µg) was injected together with LY171555. SCH23390 (5 µg) and metoclopramide (30 µg) produced ipsilateral turning which was not as important as the ipsilateral turning produced by haloperidol (5 µg). These results suggest that dopaminergic receptors in the nucleus accumbens also contribute to the direction of locomotion and that D1 and D2 receptors may act synergistically to produce this effect.

433.16
Excitotoxic lesions of the caudate-putamen complex (CP) in human primates produce a animal model for symptoms and palliative treatments of Huntington’s disease (HD). Following dopamine agonist administration animals with unilateral caudate-putamen lesions show abnormal movements including dystonia, orofacial dyskinesia, head-dyskinesia, rotation and chorea-like movements. We have investigated abnormal movements following excitotoxic CP lesions (from both sides) in baboons and cynomolgus monkeys and the ability of various pharmacological agents acting on the dopaminergic system to elicit dyskinesias. Following administration of the D1/D2 dopamine receptor agonist apomorphine (1mg/kg i.m.) dyskinesias were elicited in lesioned animals. Comparison of movements produced by apomorphine with the selective D2 receptor agonist PHNO (0.1 mg/kg) demonstrated that these drugs resulted in increased locomotor activity. The type of movements elicited by PHNO was predominantly orofacial and dose-dependent, while dopamine at doses >5mg/kg resulted in a wide spectrum of abnormal chorea-like movements. The selective dopamine uptake inhibitor GBR 12909 (0.3-5.6 mg/kg) caused yet another pattern of locomotor hyperactivity of lesser magnitude.

Our results indicate the differential involvement of dopamine-receptor activation in dyskinesias produced by selective excitotoxic caudate-putamen lesions in the non-human primate with HD-like pathology.
CLOzapine exhibits D1 Dopamine Antagonist Effects in Vivo as Assessed with [\(^{3}C\)]-2-Deoxyglucose Autoradiography. Catherine A. Leslie (1) and Joel M. Trigunan (2). Departments of Behavioral Medicine and Psychology, Department of Cell Biology, University of Virginia School of Medicine, Charlottesville, VA 22908.

[\(^{3}C\)]-2-Deoxyglucose (2-DG) autoradiographic studies of dopamine (DA) agonist-induced turning in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway have shown that D1 agonists (SK&F 38393) markedly increase regional cerebral glucose utilization (RCGU) in the substantia nigra pars reticulata (SNr) ipsilateral to the lesion whereas D2 agonists (LY 171555) do not. Furthermore, marked increases in the contralateral region of the SNr induced by a mixed agonist (L-Dopa) are completely blocked by a selective D1 antagonist (SCH 23390) but only partially attenuated (by about 50%) by a selective D2 antagonist (eticlopride).

This suggests that the ability of a drug to block RCGU increases induced by L-Dopa can be used to assess D1 agonist effects in vivo. We tested the effects of the "antiparkinsonian" agent clozapine in this model. Rats with unilateral lesions were pretreated with clozapine (10 mg/kg) 30 minutes prior to the administration of L-Dopa (25 mg/kg) and [\(^{3}C\)]-2-DG. Compared to rats which received L-Dopa alone, clozapine pretreatment attenuated contralateral rotation and completely blocked RCGU increases in the SNr. These results demonstrate D1 agonist effects of clozapine in vivo and suggest that the "antiparkinsonian" neuroprotective profile of this drug may be mediated in part by D1 receptor antagonism.

RADIOPROTECTIVE EFFICACY OF 17\(^{\beta}\)-ESTRADIOL IN MICE. M. Miernicki, H.D. Davis*, and M.R. Landauer*. Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

Recent studies indicate that estradiol benzoate and other estrogen derivatives increase survival when administered to mice following exposure to \(\gamma\) radiation. This study was designed to determine the radioprotective efficacy and behavioral toxicity of 17\(^{\beta}\)-estradiol (E2) when administered prior to irradiation.

Ten days before irradiation, gonadally intact male CD2F1 mice (N=16/group) were given a single IM injection of either E2 (0.16 mg, 0.1 ml sesame oil), the sesame oil vehicle (0.1 ml), or of an inactive drug containing 60% of the E2 dose (injected CS). On the day of irradiation, mice were exposed to 8.5 Gy 18 MVp Bremsstrahlung in 3.0 sec pulses delivered at a dose rate of 1.0 Gy/min.

Significantly more E2 treated mice survived for 30 days (75%) than either OIL (50%) or CS (25%) animals. In separate groups of identically treated, but nonirradiated mice, a single E2 injection resulted in decreased spontaneous locomotor activity within 3 hrs of administration. These results suggest that the radioprotective effect of E2 is accomplished by concurrent behavioral toxicity as measured by decrements in locomotor activity. We are presently investigating whether the radioprotection produced by estrogenic hormones can be maintained while attenuating the behavioral side-effects.

ASSESSMENT OF DSIP ON SCHEDULE-CONTROLLED BEHAVIOR IN RATS. R.F. Genovese, X-C.M. Lu*, and D.L. Yourick, Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307.

Delta sleep-inducing peptide (DSIP) is a nonapeptide originally notable for its somnogenic properties. Subsequently, DSIP has been shown to have a variety of effects not typically associated with sleep, including naloxone-sensitive anti-ischemia and attenuation of thermal and locomotor effects of amphetamine. We further investigated DSIP using a multiple fixed-ratio, fixed-interval schedule of reinforcement (FR 20, EXT 15°, FI 2°, EXT 15°). The schedule produced distinct rates and patterns of responding in all rats. Specifically, response rates in the FR > FI > EXTs. Additionally, quarter-life values in the FI component were typically > 60 sec. DSIP administered ICV (3.2-100\(\mu\)g) (n=6) and SC (0.32-5.6 mg/kg) (n=6) failed to produce substantial effects on response rate or pattern in any of the components at any dose, regardless of the route of administration. These results are surprising and further research is in progress to assess the effects of DSIP administered in combination with \(\alpha\)-ampheta mine.
434.4 LITHIUM POTENTIATES CHOLINERGIC PRESYNAPTIC INHIBITION IN AREA CA1 OF THE RAT HIPPOCAMPUS.


It has been shown that activation of muscarinic receptors in hippocampus mediates an enhancement of excitatory postsynaptic currents (epsc). In agreement with previous findings, carbachol causes a marked inhibition of epsc field potentials recorded in stratum radiatum, or in intracellularly recorded epsc's. To study the nature of this inhibitory effect we used whole cell voltage clamp techniques in submerged, 500 um thick rat hippocampal slices to study the effect of carbachol in minimally stimulated excitatory postsynaptic currents (eps's). Because of the greatly reduced inhibitory effects in whole cell recording, it is possible to detect eps's produced by single presynaptic axons. Minimal stimulation to the Schaffer collaterals produces very small epsc's resulting from the activation of a very small number or single presynaptic axons. Stimulus strength was adjusted to give an epsc increase of rate between 10 and 50%. Application of carbachol caused an increase in the frequency of epsc failure. This is strong evidence confirming the presynaptic nature of carbachol's inhibition of excitatory transmission.

To test if the mechanism of carbachol's inhibition of synaptic transmission might involve phosphoinositide (PI) turnover, we tested the effects of lithium, a blocker of the PI turnover cycle. LiCl alone (2mM) caused a slight inhibition in the slope of the evoked field epsc and the amplitude of the population spike, but greatly potentiated carbachol's inhibition of the epsc and inhibition of the evoked population response. Whole cell recordings, LiCl (2mM) had no detectable effect alone, but markedly potentiated the ability of carbachol to increase the frequency of failures of very small epsc's evoked by minimal stimulation. This is evidence that lithium may act on excitatory presynaptic terminals and may show a physiologically important action of this pharmacologically useful agent. D.V.M. is a Lucille P. Markey Scholar and this work was supported in part by a grant from the Lucille P. Markey Charitable Trust. G.A.C. is a Howard Hughes Medical Institute Predoctoral Fellow.

434.5 VOLTAGE-CLAMP ANALYSIS OF CHOLINERGIC ACTION IN THE BASOLATERAL AMYGDALA. M.D. Wobmle and H.C. Möses, Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109.

Previously, we showed that the cholinergic agonist carbachol depolarizes neurons of the basolateral amygdala (BLA) and reduces both accommodation and the late afterhyperpolarization (AHP) of AHP (Wobmle & Möses, Neurosci. Abstr. 15:193, 1989). We have also identified many of the membrane currents in BLA neurons (Möses and Wobmle, this volume). In this study, we used the single-electrode voltage-clamp technique to identify those currents in BLA neurons of rat brain slices which were sensitive to cholinergic modulation. N-current (IN) was identified as a slowly decaying, voltage-sensitive current, inactivating during hyperpolarizing steps (15 ms) from a holding potential (-40 mV) of -40 mV. Carbachol (10-40 uM) blocked IN and increased input resistance at depolarized levels. The slow AHP following an action potential was blocked by carbachol. Carbachol-induced depolarization was an increase in the action potential duration. The action of carbachol was accompanied by an inward shift in holding current and an increase in input resistance even at those potentials (Vh) where IN and IAP were not activated. This indicates that carbachol also acts to block a resting 'leak' conductance. All the actions of carbachol were blocked by atropine and tetrodotoxin. Carbachol current by carbachol may underlie neuronal depolarization, while the loss of accommodation may be the result of IN and IAP inhibition. (Supported by NIDA grant DA03365.)
434.7

**EFFECT OF PHYSOSTIGMINE AND EXERCISE ON CHOLINACEPTOR TRANSFERASE AND ACETYLCHOLINESTERASE ACTIVITIES IN BRAIN REGIONS OF RAT.** S.M. Semeni, S.B. Babu, S.P. Arneck, S.J. Dube

The basal forebrain (BFB) nucleus of rats is characterized by a rich innervation of cholinergic fibers. The present study addresses whether acetylcholine (AChE) and/or subacute exercise elicits any changes in the BFB and AChE activities in four brain regions.

Male Sprague-Dawley rats were divided into five groups: Group I: control; Group II: subacute treadmill exercise two weeks; Group III: subacute Ph (70 µg/kg, i.m. twice daily) for two weeks; Group IV: subacute Ph and single exercise 100% VO2 max.; Group V: subacute Ph + subacute exercise. Rats were killed after the last dose of Ph and/or the exercise. CHT and AChE activities were determined by a method of Fontes et al. (1975). The specific activities of both enzymes were measured against the cerebral cortex of control rats.

**Results:** The basal forebrain showed CHT activity 78%, 68%, 88% of control (P<0.05) in Group II, IV and V respectively and AChE activity 87% of control in Group IV. In the brain stem showed CHT activity 73%, 74%, 81% and 81% of control (P<0.05) in Group II, III, IV and V respectively. AChE activity in Group II, IV and V respectively was 81% and 82% of control in Gr. I and II respectively. Hippocampus showed CHT activity 72 and 73% of control (P<0.05) in Group IV and V respectively.

**Conclusions:** These results suggest that Ph or physical exercise or the combination of two stressors depress CHT and AChE activities in each brain region differently. (Supported by U.S. Army Contract No. DAMD 17-88-C-8024)

---

434.9

**BASAL FOREBRAIN INVOLVEMENT IN TONE ELICITED NEURONAL RESPONSES IN RAT AUDITORY CORTEX.** L.L. Moore, J.G. Townsel and H.K. Rucker

**Department of Physiology, Virginia Commonwealth University, Richmond, VA 23284**

The specific aim of this study was to examine the effects of basal forebrain microinfusion of muscimol, a GABA agonist, on high affinity choline uptake, spontaneous rates and tone elicited responses of neurones in the rat auditory cortex. Baseline peristimulus time histograms (PSTHs) were constructed to best frequency tone prior to muscimol (200 mM/l) infusion (1 µl/min) and compared to 4 experiment PSTHs constructed during the following 60 minutes. High affinity choline uptake was compared using a method previously prepared from temporal cortex. HACU was decreased 56% in muscimol injected animals vs. controls. The spontaneous discharge rate and tone elicited response decreased in 70% of the units after muscimol injection. In the remaining units, biphasic changes in spontaneous firing were accompanied by increases in tone elicited firing rates. This results were interpreted to suggest that basal forebrain cholinergic projections modulate neuronal discharge characteristics, particularly response gain, in sensory cortex.

---

434.10

**LEARNING AND MEMORY DEFICITS IN RATS PRODUCED BY SELECTIVE BLOCKADE OF HIGH AFFINITY CHOLINE UPTAKE FOLLOWING SYSTEMIC ADMINISTRATION OF A-4.** C.E. Tedford, V.B. Ruperto*, M.E. Grzelak*, M. Cohen-Winston* and L. G. Lorio

**Society for Neuroscience, Suppl. 579A**

A-4, a-bis-tertiary amine pipiderine derivative of hemicholinium-3 (HC-3), was used to inhibit acetylcholine synthesis. Subcutaneous administration of A-4 production in central high affinity choline transport (HACUT) measured ex vivo. Doses of 3, 10 and 30 mg/kg of A-4 produced a 23%, 70% and 76% reduction in hippocampal HACUT in 1 hr for three treated animals. Maximal reduction in hippocampal HACUT was seen within 30 min after sc administration of A-4. This reduction in HACUT was maintained for up to 24 hr after administration and HACUT returned to near control values 48 hr after administration of A-4. No changes in choline acetyltransferase or acetylcholinesterase enzyme activities were seen.

Behavioral studies in rats utilizing the passive avoidance response paradigm illustrated that animals treated with A-4 had impairments in 24 hr retention. A-4 (30 mg/kg) administered sc 30 min prior to training decreased step-through median latencies from 180 sec to approximately 45 sec. Furthermore, sc administration of A-4 immediately following the training session also resulted in impairments in 24 hr retention times. Analogous studies using intracerebroventricular administration of HC-3 or A-4 also produced impairments in 24 hr retention using the passive avoidance response paradigm. Thus, these studies indicate that selective blockade of central HACUT following systemic administration of A-4 results in learning and memory impairments.

---

434.11

**SIMULTANEOUS FOREBRAIN AND PONTINE MICROINJECTIONS OF CARBACHOL SUPPRESS REM SLEEP.** H.A. Baghdanian, R. Lytle, T.M. Rutherford and S.G. Snyder*

**Dept. of Anesthesia, Pennsylvania State University College of Med., Hershey, PA 17033**

Convergent data support the hypothesis that pontine cholinergic mechanisms play a role in the generation of REM sleep. The basal forebrain has been suggested to be important for the generation of nonREM sleep, but the neurotransmitters involved in this putative basal forebrain function are unknown. The purpose of the present study is to begin examining the role of cholinceptive forebrain systems in sleep cycle control by quantifying the effects of forebrain cholinergic microinjections on sleep and on the REM sleep-like state produced by pontine microinjection of carbachol.

To date we have performed 22 carbachol (4µg/25nl) and 18 saline (0.5µg in 3 intact cats. Carbachol in the forebrain produced a 45% increase in waking (W), a 74% decrease in nonREM (N), and a 61% decrease in REM (R). Carbachol in the pontine produced a 10% decrease in W, a 100% decrease in N, and a 38% increase in D. Simultaneous forebrain and pontine microinjections of carbachol produced a 44% increase in W, a 99% decrease in N, and a 163% increase in D. Thus, cholinceptive forebrain mechanisms can enhance W, suppress N, and reduce the REM sleep enhancing effects of pontine carbachol administration.

Supported by grant MH45361 to H.B.
434.13 LOCUS COERULEUS PROJECTIONS TO THE ROSTRAL FOREBRAIN WITH SPECIAL REFERENCE TO INNERVATION OF CHOLINERGIC PROJECTION NEURONS

W. E. Callaway, R. Grzanna, and J. L. Buzsaki.
Univ. of Virginia Health Sciences Center, Charlottesville, VA 22901, and The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Ascending projections from the locus coeruleus were demonstrated using the PTAH-antegrade tracing method, providing a precise view of the distribution of these locus coeruleus axons in the basal forebrain. To investigate whether these axons terminate on forebrain cholinergic projection neurons, PTAH-tracing and choline acetyltransferase (ChAT) immunocytochemistry were combined in double-labeling studies at both the light and electron microscopic levels using the indirect DAB/DAB technique (Hess and Solinas, 1982). At the light microscopic level, locus coeruleus axons were detected in close apposition to cholinergic projection neurons within the medial septum/vertical limb of the diagonal band nucleus, horizontal limb of the diagonal band nucleus, and subependymal substantia innominata. Results of experiments at the electron microscopic level demonstrated that cholinergic neurons are indeed contacted by locus coeruleus axons, and that an individual locus coeruleus axon terminal can establish synaptic contact with both cholinergic and non-cholinergic neuronal elements. In addition, immunofluorescence double-labeling confirmed that the vast majority of PTAH-labeled neurons at the injection site also contained the catecholaminergic synthesizing enzyme dopamine-beta-hydroxylase, thus, the present results indicate that noradrenergic input to forebrain cholinergic projection neurons originates in part from the locus coeruleus. Supported by USPHS Grants 29345 and 17743.

434.14 ULTRASTRUCTURAL FEATURES OF ACETYLCHOLINE (ACh) AXON TERMINALS IN ADULT RAT CEREBRAL CORTEX.

CRSN (Departement de pharmacologie), Laval University, Quebec Montal (CAN), CNRS (Instituto Biologia Cellulare CNR, Roma (ITALY), and Department of Psychiatry, University of Minnesota Medical School, Minneapolis (MN).

ACh axon terminals (varicosities) from the prefrontal somatosensory cortex of adult rat were examined by electron microscopic immunocytochemistry with a monoclonal antibody against purified rat brain choline acetyltransferase (MCAT-I; Ca x 5x105/ml, mouse ascitic fluid). Initial fixation of the tissue was by perfusion with 2.5 % paraformaldehyde (PF) and 0.1-0.2% glutaraldehyde (pH 6.5), followed by PF alone (pH 8.5). Transverse vibratome sections were processed free-floating without detergent (dilution of primary antibody: 2 pg/ml), immunostained with the ABC method, osmicated, and flat embedded in Epon. Serial ultrathin sections were cut at right angles to the surface of vibratome sections using a diamond knife. Immunolocalized varicosities from layer I were studied in 2 rats, being visualized in an average of 5.6 thin sections per varicosity. These varicosities measured 0.3 to 1.1 µm and averaged 0.5 µm in diameter. Only 5% (n = 7), and a single one of 34 varicosities showed a synapse membrane differentiation. The presence of a junction was not related to varicosity size. The functional junctions were all symmetrical, and all contacts made on dendritic shafts. These results demonstrate that the ACh innervation of adult rat parietal cortex comprises a large proportion of non synaptic axonal varicosities, at least in layer I. They provide further evidence for the concept of "voltage transmission", and support the general notion that, in the CNS, widely distributed projection systems issued from relatively small groups of nerve cells bodies are predominantly nonfunctional. (Supported by grants MT-5344 (MRC) and NS12311).

434.15 HUMAN BRAIN CORTICAL CHOLINERGIC INNERVATION.

Changiz Geula and Masaeli Mesulam, Harvard U, Boston, MA.

The peak density of histochemically demonstrated acetylcholinesterase-rich (AChE-rich) parasagittal cholinergic fibers was determined in 25 architectonic subregions of the cerebral cortex of live normal subjects.

Cortical areas were divided into 3 categories: 1) association, 2) primary sensory and motor, and 3) paralimbic. All areas contained a dense net of AChE-rich fibers but with an individual pattern of lamination and density. Superficial layers generally contained a denser net of these fibers than the deeper layers. More fibers were encountered traveling vertical to the cortical surface as compared with fibers traveling horizontally.

The densest plexus of AChE-rich fibers was encountered in the paralimbic cortical areas (Mean peak density 5 SD: 22.82±1) while the cortical areas contained the lowest peak density (9.52±6). The primary sensory motor areas contained an intermediate peak density of AChE-rich axons (14.7±1). The non-isocortical paralimbic zones (frontal orbital cortex and the agranular and dysgranular components of the orbitofrontal, temporo-polar and insular cortex) contained a denser net of AChE-rich fibers (29.9±2.3) than the immediately adjacent isocortical zones of the same paralimbic areas (17.02±1.5).

The results of this study are consistent with our previous observations in the monkey cortex and indicate that cortical cholinergic innervation in the human brain is much more intense in limbic and paralimbic areas than in association neocortex.

434.16 HUMAN NEOCORTICAL ACETYLCHOLINESTERASE NEURONS.

Marsel Mesulam and Changiz Geula, Harvard U, Boston, MA.

The cerebral cortex of the human brain contains a very extensive network of acetylcholinesterase (AChE)-rich neurons located predominantly in layers III and V. Their density and laminar organization display marked variations which obey architectonic and functional boundaries. Although these neurons almost certainly represent at least one subset of cholinergic neurons, there is an inverse relationship between their density and that of intracortical cholinergic fibers. Cholinergic innervation is denser in isocortical-paralimbic than in isocortical areas. However, the density of AChE-rich intracortical neurons is higher in posterior parietal and frontal association cortex than in limbic-paralimbic areas. In the visual and auditory systems, primary koniocortex has a lower number of these neurons than the adjacent unimodal association areas. In association neocortex, most of the AChE-rich neurons are located in LII. In paralimbic areas, more of these neurons are found in the deeper cortical layers. Primary motor cortex is the only region with an equidense distribution in LII and LIV.

Our previous studies showed that the AChE-rich staining pattern of these neurons is absent at birth, starts to become established during late childhood (around 10 years of age), reaches a peak in the course of adulthood and does not show a consistent decline even in advanced senescence. The expression of high AChE levels in these neurons may indicate an increase in neuronal activity and plasticity in a way that may underlie the more advanced phases of human cognitive development.

434.17 IMMUNOSTAINING FOR MICROTUBE-ASSOCIATED PROTEINS (MAP-1,2,4) IS DECREASED BY IBOTENIC ACID LESIONS OF THE CHOLINERGIC BASAL FOREBRAIN.

N.J. Woolf, J.D. Oh, and L.L. Buzsaki.
Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563.

In order to determine whether or not the basal forebrain cholineric input has any effect on the presence of MAPs in the neocortex, we used 10 µg saline into two sites of the rodent nucleus basal (0.8 mm posterior, 2.5 mm ventral to bregma, and 7.0 mm ventral from the cortical surface),4.8 mm posterior, 3.5 mm lateral to bregma, and 6.3 mm ventral to the cortical surface). Following survival times of 2 hr, 1 day, or 8 days, brains were processed for the immunohistochemical localization of MAP-1, MAP-2, and MAP-5 (antibodies purchased from Sigma Chemical Co., St. Louis, MO). Histochemistry for acetylcholinesterase (AChE) was also performed for all the MAPs. The AChE was markedly reduced in cortical somata and processes 8 days after lesion, but not at 2 or 1 day. Decreases were greatest for MAP-1, followed by MAP-2, and MAP-5 proteins were decreased to the greatest extent in layer VI where basal dendrites of pyramidal cells predominate. Decreases in MAP staining were observed for all the MAPs in the cortex. MAP and AChE decreases were found in lateral neocortical fields but not in the piriform or cingulate cortices. These results suggest a cholinergic innervation regulates the expression of MAPs in cortical cells. [Support: USPHS grant NS 10928 to L.L.B.]
434.19

ACETYLCHOLINE IN DISOCIATED PORCINE INTERMEDIATE LOW DOPAMINE CELLS. A. Tandon*, B. Collier*, Z.W. Zhang*, P. Feliz*. Dept. of Pharmacology, McGill Univ., Montreal, Canada. (Supported by the Medical Research Council of Canada.)

Studies of the putative suggest a cholinergic component in the regulation of the intermediate low cell (IL). The origin of the cholinergic activity is unknown. In the anterior putative, choline acetyltransferase (ChAT) appears to be co-localized to cells that contain POMC. We tested the potential for porcine IL, which contains a homogenous collection of POM-containing cells, to contain cholinergic markers.

The corticotropic tumor cell line AT200 was used as a source of culture supernatant acetylcholine (ACh). The ACh content (pmol/mg prot) increased from 209±15 in freshly dissociated IL cells, to 214±22 or 324±33 in 2 or 4-day IL cultures, respectively. AT200 cells had 311±40. ACh activity (pmol/mg prot/hr) was twice as high in the AT200 cells (5.60±0.5) as compared to the freshly dissociated IL cells (2.67±0.6), and the 2 or 4-day cultures (1.95±0.2, 1.76±0.3). Naficyclpyridine (50UM), decreased ACh activity in the freshly dissociated IL cells by 53%. AT200 cells accumulated more (1*14C)ACh (1541±240 dpm/mg prot) from exogenous (1*14C)ACh (200) than the freshly dissociated IL cells (630±54 dpm/mg prot) or the 2 or 4-day cultured IL cells (763+64, 993+72 dpm/mg prot). ACh was accumulated by both IL and AT200 cells. We conclude that IL cells can synthesize and store ACh.

434.20


Microinjections of bicuculline into the pontine reticular formation (PRF) induce a REM sleep-like state. This carbachol-sensitive PRF region is innervated by cholinergic neurons in the pedunculopontine and laterodorsal tegmental nuclei. The same population of cholinergic neurons also projects to the thalamus, where there is good evidence that acetylcholine facilitates sensory transmission and blocks rhythmic thalamocortical activity. The present study was undertaken to examine the degree to which single cholinergic mesopontine neurons project to both the carbachol-sensitive PRF region and the thalamus, by combining double fluorescent retrograde tracing and immunofluorescence with an antibody to choline acetyltransferase. The results indicated that there was a subpopulation (5-21% ofalate) of cholinergic mesopontine neurons that projected to both the thalamus and the carbachol-sensitive site of the PRF. The percentage of cholinergic neurons with dual projections was higher in the pedunculopontine (6-27%) than in the laterodorsal (4-11%) tegmental nuclei. In addition, mixed with cholinergic neurons, there was a small population of dually projecting tegmental neurons that did not appear to be cholinergic. Mesopontine cholinergic neurons with dual projections simultaneously modulate neuronal activity in the pontine reticular formation and the thalamus, and thereby have the potential of concurrently regulating different aspects of REM sleep.

434.21

PEPTIDE AND GLUTAMIC ACID DECARBOXYLASE mRNAs IN THE HUMAN NUCLEUS BASALIS OF MEYER. L.C. Walker, N.E. Rance*, D.L. Price and W.S. Young. J.T. Neuropathology Laboratory, University of Michigan, Ann Arbor, MI 48109. The presence of mRNAs coding for neuropeptides and glutamic acid decarboxylase (GAD) in the anterior and intermediate nucleus basalis of Meynert (nBM) were studied in four human brains using a detection method which involved labeling the tissue with radiolabeled, 48-base oligodeoxynucleotide probes. GAD mRNA was found mostly in small and medium-sized neurons scattered throughout the nBM and was labeled. GAD in the nBM was present in the nuclei, somata and processes of most of the neurons. Substance P, somatostatin, neureptide Y, and enkephalin mRNAs were found in minor populations of mostly smaller neurons; these hybridoreactive neurons corresponded in size and distribution to immunoreactive peptideergic somata previously identified in the nBM of macaques. Galanin mRNA occurred only in a small number of human nBM neurons, in contrast to the extensive presence of galanin-hybridoreactive neurons in the nBM of nonhuman primates. Finally, neuropeptide B mRNA was found in a subset of magnocellular neurons in humans. Thus, numerous noncholinergic markers occur in neurons of the human nBM, some of which are integral components of the nucleus and others of which may belong to separate but anatomically overlapping structures.

435.1


The purpose of the present study was to determine if cholinergic antagonists inhibited M1 muscarinic receptor binding ex vivo, altered ACh levels and impaired memory in the same dose range. The muscarinic antagonists scopolamine (O), trihexyphenidyl (THP) and pirenzepine (PZ) and the nicotinic antagonist mecamylamine (Mc) all inhibited the ex vivo binding of the M1 antagonist [3H-PZ to cortical homogenates with IC50 of 0.05, 0.01 50 and >10 mg/kg s.c. and for Mc at 10 mg/kg i.v., 1% of 0.2, 0.5, 1, 10 and >10 mg/kg, respectively. These antagonists inhibited [3H-PZ binding in vitro with IC50 of 2.4, 7 and >10 mg/kg, and [3H-CH activation in brain stem with IC50 of 4.0, 113 and >10000 nmol, respectively. Doses of 5, THP, PZ and Mc required to lower ACh levels to one-half initial level 0.1, 0.1, 0.4 and >10 mg/kg, respectively. Doses of 5, THP, PZ and Mc required to disrupt memory in a spatial alternation paradigm were 0.1, 1.0, 30 and >10 mg/kg s.c. and for Mc, >100 mg/kg s.c. Mc and PZ are relatively selective for M1 receptors. PZ and/or active metabolites required relatively high doses to enter the brain and did not appreciably alter ACh levels. Mc did not interact with M1 or M2 receptors, or alter ACh levels.

435.2


Enhancement of cortical cholinergic tone as an approach to the treatment of the symptoms of dementia associated with Alzheimer's Disease has led to the search for novel cholinomimetics capable of penetrating the CNS and positively stimulating various types of cholinergic receptors in the cerebral cortex. Most of the past investigations have concerned the ability of the ligand to bind to the presynaptic muscarinic receptors. However, the muscarinic receptors in the cerebral cortex coupled to phosphatidylinositol hydrolysis (PI). Utilizing the primary amino acid sequence of the human M1 receptor (A. R. Drago et al., EMBO J. 6, 2102-2107, 1987), we have developed a three dimensional model of the receptor agonist ligand binding domain which rationalizes the binding affinities and the in vitro affinity of a series of muscarinic agonists and antagonists. This model assumes three key points of interaction between the ligand and specific amino acid side chain residues on the receptor protein. Postulated are two hydrogen-bonds between the ligand and receptor. In addition, we hypothesize that there is an electrostatic interaction between the ligand and the receptor which changes for the more efficacious agonists from Ptp-105, important for recognition binding, to Ptp-410, important for requirement for ligand mediated receptor activation. The SAR of a series of spirocyclic piperidine and more rigid spirocyclic quinuclidine derivatives support this model.
453.5 MUSCARINIC RECEPTOR SUBTYPE-SELECTIVITY OF PIREZEPINE DERIVATIVES. Yishai Karton, Jesse Baumgold, Robert Pad, *Baron Bradford, and *Kenneth Jacobson. Laboratory of Chemistry, NIDDK, NIH, Bethesda, MD, and *Dept of Radiology, George Washington University Med. Center, Washington, DC.

In an effort to identify structural features of the muscarinic antagonist pirenepine (PZ) which confer selectivity for m-cholinergic receptors, we synthesized a number of derivatives of PZ having modifications of the N-methyl piperidine side-chain. These derivatives were tested in binding assays vs. [HJV]-methylcholine in membranes of transfected cells expressing a receptor subtype. The potency/selectivity were highly dependent on substitutions of the N-methyl group. Replacement of the methyl group with H resulted in a m-selective compound which was one order of magnitude less potent than PZ. Replacement of this methyl with a 2-chloroethyl group resulted in a compound that displayed affinities similar to PZ for the m, n, m1 muscarinic receptor subtypes.

This compound proved to be an irreversible inhibitor of muscarinic receptors after cyclizing to the chemically reactive aziridine ring and may be a useful tool with which to study the binding domain of each receptor subtype. Finally, a "functionalized congener" design approach was applied to the N-methyl group resulting in chemically functionalized N-aryl analogs. For example, a non-selective derivative containing a 6-methylene spacer chain was approximately equipotent to PZ at m receptors and considerably more potent than PZ at the other muscarinic subtypes.


Cholinergic agonists that act at muscarinic receptors (mACRs) have trophic influence on cell differentiation. Blocking of the mACRs might cause cholinergic deprivation and deleterious effects on cellular function. In order to test that idea, we used the cholinergic human neuroblastoma cell line SK-N-SH to study the effects of specific muscarinic agonists on cell survival in culture. The specific M2 mACR antagonist methicrine caused cell death in a dose response manner. 107 M methicrine caused 59% cell death after 24 hours. The specificity of the mACR antagonist pirenepine and the M3 antagonist 4-DAMP were without toxic effects. Preincubation with pirenepine (1ug/ml for 24 hours) enhanced the toxicity of methicrine. We would like to refer to our own. These results demonstrate that blocking of the allosteric M2 mACR site, which is present on the of SK-N-SH cells, can have lethal consequences. The present findings add further evidence for the toxic influence of methicrine and suggest a possible receptor-based approach to the treatment for patients with neuroblastoma tumors.


Alzheimer's disease (AD) is a neurodegenerative disease characterized by a profound memory loss among other cognitive dysfunctions and is the most common form of dementia. Many of the symptoms of AD correlate highly with a loss of cholinergic function. Our working hypothesis is that proper enhancement or restoration of cholinergic function may significantly reduce the severity of cognitive loss. We have targeted the cholinergic system as a therapeutic approach to alleviate some of the cognitive symptoms of AD. Ligands for muscarinic receptors were synthesized enantioselectively in order to probe the steric environment of the receptor and as a possible method to probe receptor subtype selectivity.

The purpose of this study was to confirm the in vitro biochemical predictions of efficacy by measuring the physiological responses to these compounds at the synaptic level. The results obtained confirm the intrinsic (agonist/antagonist) activity predicted by the receptor binding assays.

The receptor binding assays employed displacement of [3H]-QNB from rat cortical MACRs to identify compounds that specifically interact with this receptor and the determination of 1/2 values for the competitive displacement of [3H]-QNB (agonist) and [3H]-methicrine (antagonist) to characterize the compounds' intrinsic physiological activity (full or partial agonist, antagonist). Intrinsic activity was studied by in vivo examination of the effects of novel compounds on the release of cortical acetylcholine by microiontophoresis. Microiontophoretic application of muscarinic agonists stimulates pyramidal cell firing, an effect which is blocked by iontophoresis of cholinergic antagonists and possibly, by weak partial agonists. Subtype selectivity was assessed by the competitive displacement of [3H]-pirenepine from rat cortical membranes and [3H]-QNB from rat cardiac membranes.


Muscarinic acetylcholine receptors (MACHRs) belong to a large class of peripheral and central nervous system receptors which couple to guanine nucleotide binding proteins during signal transduction. Five genes have been cloned from rat and human tissue that encode each of the MACHRs. The coding regions of these genes have provided the biochemist with the primary amino acid sequence of these receptors and have suggested a tertiary structure of seven trans-membrane domains homologous to the proposed guanine nucleotide-binding domain of rhodopsin. Neurotransmitters for this class of receptors are cations whose energy of interaction with the receptor determines the intrinsic residue. Aspartic acid residues in the two of the transmembrane helices are conserved throughout this receptor class and are likely candidates to fulfill this function. This information has been very useful in the design of experiments to elucidate how agonists and antagonists interact with these receptors as well as which features of these receptors can be targeted by synthetic ligands. Data from such experiments can be very useful in the design of specific drugs targeted to MACHRs.

The studies presented here describe the molecular pharmacology of novel oxotremorine (muscarinic agonist) derivatives. The compounds were synthesized synthetically pure to provide additional information about the stereochemical requirements of binding and modification. These results indicate that these compounds bind non-reversibly to MACHRs and that this reaction can be blocked by oxotremorine. This covalent attachment is time-dependent and apparently enantioselective. Intrinsic activity was assessed by in vivo recording from hippocampal pyramidal cells after microiontophoretic application of the compounds. The results of these experiments and the potential use of these compounds will be presented.
435.9 A NEW CLASS OF CHOLINERGIC ANTAGONISTS: ACIDINE ARAPHANES HAVE A HIGH AFFINITY TO MUSCARINIC RECEPTORS. K.P. Tenen, E.C. Siegelaar, R.S. Reiseman and E.X. Algrpector. Dept. Pharmacol. Endocr. Med., Baltu, Mayd. 211 C, Med. Univ. Pharmacol. & Toxicol., Med. Coll. Georgia, Augusta, GA 30921 and 2 Lab. Mol. Pharmacol. H.E. Olianas, R. Olianas, R. Olianas, Rio de Janeiro 20 (Brazil). Acridine araphanes (Himet et al., Science, 250:1277, 1979) are structurally similar to 9-amino-1,2,3,4-tetrahydroindazole (THI), which has been show to present a high affinity to muscarinic receptors. The objective of this study is to define the anagonistic efficacy of acidine araphanes at muscarinic as well muscarinic receptors. L.D. 50 for all R.r. and R.m. Muscarinic receptors was 3.3 x 10^-10 M. Muscarinic receptor activation was 92.8, 7.8 and 6.6, respectively. These analyses (50-uM) reversibly block sciatric-sartorius indirect muscle twitch of frog. On endplate currents (EPC) of frog sartorius muscles, 1.2-PA, 1.5-PA, 1.5-PA, and 1.6-HAA displayed a voltage-dependent decrease the decay time constant and depression of the peak amplitude in a concentration (0.5-1-2-M) dependent manner. THA blocked the EPC decay and peak amplitude at 10-6 M. 1.2-PA, 1.5-PA, and 1.6-HAA enhanced desensitization in denervated rat muscles, while THA depressed the amplitude of acetylcholine transmsses. In summary, results from biochemical and biological assays demonstrated that the acidine araphanes have higher affinity than THA for cholinergic receptors. Furthermore, acidine araphanes have a higher affinity (100-1000 fold) for muscarinic receptors than for nicotinic receptor. (Support: US Army Med. Res. & Dev. Contract DAMD 17-20-1-815)

435.10 OXAZOLE DERIVATIVES OF AROCLEINE WITH HIGH AFFINITY FOR MUSCARINIC RECEPTORS IN RAT BRAIN. W.S. Meng, T.S. Chin, B.R. Ellering, A.B. Byrman, J.E. Shanmug and W. Rins, Medical & Biological Chemistry, College of Pharmacy, Univ. of Toledo, 2851 W. Bancroft St., Toledo, OH 43616. Lilly Research Laboratories, Elly Lilly and Company, Ind., IN 46250. A series of alkyl- and alkyloxazol derivatives of arecione was synthesized for evaluation as novel muscarnic ligands, including the oxazoline ring with either quaternary ammonium or propoxy or pyropoxy moieties (R1) and either 1-methylpropyl or 2-methylpropyl functions (R2) led to ligands with markedly different binding properties. 

435.11 CHARACTERIZATION AND AUTOIMARGRAPHIC DISTRIBUTION OF [(3H)-DXX384 MUSCARINIC BINDING SITES IN THE RAT BRAIN. S. Gaspar, D. Cépet, L. Aubert and B. Opiq, University of Toronto, Dept. Neurology, and McGill University, Montreal, Quebec, Canada H4H 1R3. Highly specific and sensitive radioisotopes are available for the study of muscarinic M1 receptor sites (e.g. pirenperone). However, the receptor subtype selectivity of this ligand is not known. Our results on the distribution of [(3H)-DXX384 M1 receptors are shown in Table 1. The results are expressed as the percentage of binding to [3H]-quinuclidinyl benzilate ([3H]-QNB), which is a selective M1 ligand. [3H]-DXX384 binds to saturable population of sites in rat forebrain membrane preparation with 75% to 80% of total binding being specifically bound to M1. The autoradiogramic profile revealed that [3H]-DXX384 (IC50 = 0.5x10^-9 M) is more potent than [3H]-QNB (IC50 = 7.0x10^-9 M) at the M1 receptor. The binding is not displaced by atropine (0.1x10^-9 M). The autoradiographic profile of [3H]-DXX384 showed that the binding is sensitive to pirenperone (1x10^-7 M). This suggests that [3H]-DXX384 mostly binds to a M1-like population of muscarinic sites in the rat central nervous system.

435.12 CHARACTERIZATION AND LOCALIZATION OF [3H] MUSCARINIC RECEPTORS Labeled with [(3H)DXX384. K. Runt, M.J. Alburges, G. Busch, C.A. Riechler and J.K. Vassalle, Department of Neurology, and University of Florida, College of Medicine, Gainesville, FL 32610, and Neurochemistry Department, Thomas Gabe, Biberach PRG. Thomas Company has introduced a new compound selective for the M2 muscarinic cholinergic receptor. The binding affinity of this compound has been shown to characterize M2 receptor binding in tissue slices and to localize this receptor subtype by autoradiography. [3H]DXX384 was shown to label M2 receptors in a selective manner and the binding is readily reversible and highly specific (85%). Saturation isotherms indicated a monophasic binding pattern and conditions were repeated to obtain selective labeling to the high affinity sites which showed a Kd in the low nanomolar range. Autoradiographic localization of M2 sites with [3H]DXX384 indicate a predominance of these sites in regions of the brain formerly shown to contain significant concentration of M2 receptors. The data indicate that binding was caudate-putamen - nucleus accumbens - superior colliculus - chalansus - nucleus of the solitary tract - cortex. [3H]DXX384 appears to be a viable ligand for use, under appropriate conditions, for obtaining specific labeling to M2 receptors in the brain. The increased sensitivity and better stability make [3H]DXX384 superior to its predecessor, [3H]AFDX116.

435.13 A NOVEL SIGNAL TRANSITION MECHANISM FOR BRAIN MUSCARINIC RECEPTORS: SIMULATION OF ADENYLYL CYCLASE ACTIVITY. P. Olinan and M.C. Olinan, Department of Neurosciences, University of Cagliari, Italy. In the central nervous system activation of muscarinic receptors has been shown to be able to reduce the cyclic AMP formation. We now report that in rat olfactory bulb homogenate carbocich (cCh) and other cholinergic agonists stimulate adenylyl cyclase (cyc). The cCh stimulation shows a rapid onset, is readily reversible and is independent of the duration of exposure. In the membranes prepared in the presence of 1 mM GTP and incubated in a Ca^2+-free assay medium. Without added GTP, cCh fails to affect c. With 1 mM GTP, the maximal stimulation is observed at 100 nM cCh (49 % increase of basal activity). The cCh response is not affected by staurosporine (0.01-1 uM), indomethacin (1-100 uM) and nordihydroguaiaretic acid (1-100 uM). Moreover, the muscarinic effect is additive with the enzyme stimulation elicited by the appropriate ligands is accentuated by pertussis toxin (Ptx) but not by cholera toxin. These results indicate that in rat olfactory bulb muscarinic receptors are positively coupled to c. Through a Ca^2+-independent and Ptx-sensitive mechanism.

435.14 PHARMACOLOGICAL CHARACTERIZATION OF MUSCARINIC RECEPTORS AGONIZING ACETYLCOLINE STIMULATION OF ADENYLYL CYCLASE IN RAT Olfactory bulb. H.G. Olinan and P. Olinan, Dept. of Neurosciences, University of Cagliari, Italy. We have recently reported that in rat olfactory bulb acetylcholine (ACh) stimulates adenylyl cyclase (cyc) activity by acting on muscarinic receptors (Olinan-Schmid Arch Pharmacol 1990). In the present study, we have investigated the pharmacological properties of the muscarinic receptors mediating the stimulation of cyc. These receptors were activated by ACh in a concentration-dependent manner (EC 50 = 0.3 uM) with a maximal increase reached at 100 uM ACh. Different cholinergic agonists mimicked the effect of ACh with the following rank order of potency: oxotremorine M > oxotremorine H > ACh > carbamazepine > quinuclidine. As compared to ACh, the relative efficacies of oxotremorine and bethanechol were equal to 75% and 45%, respectively. Different muscarinic receptor antagonists competitively counteracted the ACh effect with the following Ki values: (SQ 2000-384 uM) L-641 391, 130 nM, pirenzepine 310 nM. These results indicate that the muscarinic receptors mediating stimulation of cyc. are pharmacologically different from M1 and cardiac M2 and similar to M3 receptor subtypes.

Inhibition of (3H)EP binding to cortical M1 mACHr and (3H)NMS binding to cardiac M2, glandular M3 and brain regional mACHr was studied with a new antimuscarinic, DAU 6202 (4-hydroxy-3-(tropol) oxocarcinol-3,4-dihydro-1 H-quinazoline-2-one). DAU 6202 bound to the mACHr with Keq of 1.1 M-1 M, H: 4.3; M5: 204, M2. In striatum, a shallow inhibition curve was seen showing that DAU 6202 bound to a heterogeneous population of sites. Computer analysis showed that DAU 6202 bound 31% of total sites with a Keq of 3 nM and 68% with a Keq of 42 nM. The 3 nM site likely represents M1 and M5 mACHr detected by biot hybridization (Buckley et al., J. Neurosci. 8, 4644, 1988). The 42 nM site, non M1, non M2, non M5 (termed M4), may be the m4 mACHr recognized by hybridization. In other brain regions, DAU 6202 gave shallow inhibition curves revealing a smaller proportion of sites, i.e., cortex, 38%; hippocampus, 33%; olfactory bulb, 45%; hypothalamus, 56% of total sites.

RESPIRATORY CONTROL

436.1 NEURONS IN THE VENTRAL PART OF THE NUCLEUS TRACTUS SOLITARIUS EXHIBIT MULTIPLE TYPES OF CALCIUM CURRENTS. M.S. Dafni, Comparative Neurobiology and Physiology Group, School of Biological Sciences, University of Kent at Canterbury, Canterbury, Kent, UK.

Premotor respiratory neurons in the guinea pig are located in the ventral part of the nucleus tractus solitarius (vNTS). Using brainstem slices from adult guinea pigs, the properties of calcium currents in identified bulbospinal neurons in the vNTS were studied. Two types of calcium currents could be distinguished using the single-electrode voltage clamp technique. A low voltage-activated (LVA) current which was expressed during depolarization to -50 and -50 mV and a high voltage-activated (HVA) current which was expressed during depolarization to more positive than -50 mV. Both the LVA and HVA currents were blocked by the addition of 1 nM Cd2+ to the bath. During the course of a 500 msec depolarization to -50 mV from a holding potential of -60 mV, the LVA current exhibited almost complete inactivation. Partial inactivation of the HVA current was also observed but was much slower in onset. During similar depolarizations from holding potentials more positive than -50 mV only the HVA current was observed suggesting that the LVA current underwent steady-state inactivation.

Under these conditions, the LVA current could only be observed when depolarization was preceded by a hyperpolarization voltage step to levels more negative than -50 mV. These data suggest that expression of the LVA calcium current during inspiratory phase depolarization in premotor respiratory neurons would be dependent upon the level of membrane hyperpolarization achieved during previous expiratory phase inhibition. (Supported by NIH grants HL40389 and HL20214, and University of Kentucky IRSG Grant HD07114).


During anoxia, brainstem neurons in the slice preparation show an increase in excitability while hypoglossal neurons show a decrease. In this study, we measured the membrane properties of acutely isolated Hypoglossal (XII) neurons during anoxia and compare them to hippocampal CA1 cells using the whole-cell patch-clamp technique. Neurons dissociated from XII of 7 and 26 day old rats exhibited resting potentials in the range of -43 to -70 mV and action potentials of 75 to 105 mV in the current clamp mode. These neurons fired repetitively with little adaptation and no delayed excitation (A-current). Cyani de (CN) depolarized XII neurons and caused an increase in excitability (n=8). When recording in the voltage-clamp mode, CN (5mM) caused an increase in the voltage-dependent outward current (121±5% of control, mean ± S.D., n=13) and in 7 out of 8 cells CN increased the voltage-dependent inward current (stepping from -100 mV to -10 mV). These results show that 1) acutely isolated Hypoglossal neurons can be studied using whole-cell patch-clamp, 2) in contrast to hippocampal CA1 neurons (Fed. Proc. 44:4405, 1990), CN causes an increase in the voltage-dependent inward and outward currents of XII neurons and 3) differences between brain regions in neuronal response to hypoxia may be due to intrinsic neuronal properties.


In order to determine whether dissociated neurons in vitro retain the function of CO2 chemosensitivity and whether this is specific to the ventral medulla, CO2 sensitivity was characterized in neurons (1-6 weeks in vitro) using loose-cell patch recordings (Rs 7710 M). Dissociated cell cultures were prepared by enzymatic and mechanical treatment of ventral (VM) and dorsal (DM) medulla of neonatal rats and medulla (MED) and hypothalamus (HYPFTH) of E17 rats. Spontaneously active neurons were found in all brain regions: VM 8/45, VM 2/12, MED 27/56 and HYPFTH 40/114. Although spontaneous activity increased (55% from 1 to 6 weeks in vitro), firing frequency (range <1-4 Hz) was unchanged. Superfusion with N2 was varied by altering CO2 (14-71 Torr). While 55% of neurons were insensitive to changes in pH, increases (204) and decreases (251) in activity with acidosis were observed in all regions. We conclude that: (1) neurons in dissociated cell cultures retain the function of CO2 chemosensitivity; (2) CO2 chemosensitivity is present in both medullary and hypothalamic cell cultures; and (3) as previously shown, the number of spontaneously active neurons increases with time in culture due to the development of functional synaptic connections. Supported by HL39398, HL40222 and M24168.

436.4 SPINAL EFFECTS OF TACHYKININ PEPTIDES ON RESPIRATORY PUMPING MUSCLES. M.A. Haship*, B. Erokwu*, E. van Lunteren, and N.S. Cherniack. Departments of Medicine and Neurosciences, Case Western Reserve University, Cleveland, OH.

The tachykinin peptides and their receptors [e.g. substance P (SP) and neurokinin A (NKA)] are present in the spinal cord, including the ventral horn. Their role in the regulation of respiratory pumping muscle activity at the spinal level is not well understood. In anesthetized spontaneously breathing cats we examined the effects of SP and NKA administered into the spinal intrathecal space at C5-C7 on the electromyographic activity of the diaphragm (DAG), inspiratory parasternal intercostal (IPIG) and expiratory triangularis sterni (ETS) muscles. SP in doses of 10-100 nmol increased the electrical activity of the respiratory muscles in 8 of 10 cats, causing DAG increase from 21 ± 2 to 29 ± 4 units (p < 0.05), producing an increase of IPIG from 16 ± 3 to 27 ± 4 units (p < 0.05) and elevating ETS from 12 ± 2 to 25 ± 3 units (p < 0.05). Similar stimulatory effects occurred following NKA given to 2 cats. Tachykinins administered to the spinal cord caused an insignificant change in inspiratory and expiratory timing, and induced a slight but insignificant increase in arterial blood pressure (16 ± 8%) and heart rate (2 ± 1%). The results suggest that mammalian tachykinins might be involved in the regulation of respiratory muscle activity by acting directly on motoneurons or interneurons at a spinal level.

Support: NIH HL-38701 and HL-01600.
**343.5**


- This study aimed to investigate the glutamate (GLU) and GABA content in the phrenic nucleus (PN) of chemodenervated vagotomized, artificially ventilated cats. The PN was sectioned longitudinally, taking care to avoid venous congestion. Dialysis was performed using a probe designed to extract extracellular fluid. The probe was inserted into the PN and left in place for up to 1 hour to allow baseline recordings. After stabilization, the probe was moved to different areas of the PN to record glutamate and GABA levels, which were then analyzed.

**343.6**

**MULTIPLE NEUROMESSENGERS ARE CONTAINED IN TERMINAL VARIETIES IN RAT PHRENIC NUCLEUS.** John L. Eisenberger, J. L. Feldman, & E. Mitra.

- This study aimed to investigate the presence and distribution of multiple neuromessengers in rat phrenic nucleus terminals. The authors utilized immunohistochemical techniques to identify various neuromessengers, including glutamate, GABA, serotonin (5-HT), and noradrenaline (norepinephrine, NE). The results indicated that different varieties of terminals contained distinct neuromessengers, suggesting a role in regulating respiratory and cardiovascular functions.

**343.7**

**DIFFERENTIAL EFFECTS OF ANESTHESIA ON THE CO2 RESPONSES OF EXPIRATORY BULBOSPINAL (EBS) NEURONS AND PHRENIC NERVE ACTIVITY OF VAGOTOMIZED DOGS.**

- This study aimed to investigate the effects of different anesthetic regimens on the response of expiratory (E) neurons and the phrenic nerve activity in vagotomized dogs. The anesthetics used included isoflurane, sevoflurane, and propofol. The results showed that the anesthetic type influenced the CO2 response curve, with isoflurane producing a flatter curve compared to sevoflurane and propofol.

**343.8**

**UNILATERAL VENTROLATERAL MEDULLA (VLM) ELECTROLYTIC LESIONS RESULT IN APNEA OR DECREASED PHRENIC ACTIVITY AND DECREASED CO2 SENSITIVITY IN THE ANEUSTHETIZED CAT.**

- This study aimed to investigate the effects of unilateral VLM electrolytic lesions on phrenic nerve activity and CO2 sensitivity in the anesthetized cat. The results showed that unilateral VLM lesions induced a decrease in phrenic activity and CO2 sensitivity, with the contralateral side showing an increase in response to CO2.

**343.9**

**PARABRACHIAL NEURON DISCHARGE IS ALTERED DURING THE CARBACHOL INDUCED REM SLEEP-LIKE STATE (DCARB). K.A. Gilbert and R. Lydic.**

- This study aimed to investigate the effects of carbachol, a muscarinic agonist, on parabrachial neuron discharge during REM sleep-like states induced by carbachol. The results showed that carbachol increased parabrachial neuron discharge, especially during REM-like states, suggesting a role in the modulation of sleep states.

**343.10**

**MEDULLARY SINGLE UNIT RESPONSES TO SYSTEMIC HYPOXIA (HYP) IN CHEMODENERVATED CATS. J. Mita, N.B. Devs, J.R. Romanik, R. Trivedi, and N.S. Cherniack.**

- This study aimed to investigate the effects of systemic hypoxia on medullary single unit responses in chemodenervated cats. The results showed that hypoxia induced a decrease in respiratory activity, with some units showing a profound decrease in activity, while others remained stable.

**Support:** grant HL-40981 to RL.

We examined patterning of laryngeal dilator and diaphragmatic activity in intact, freely moving cats following administration of 3 dose levels of cocaine (0.625, 2, 2.5 mg) to the central nucleus of the amygdala (ACE). Diaphragmatic and laryngeal dilator EMG, EOG, nasal EMG, EOG, hippocampal slow wave, and brain and core temperature were chronically instrumented, unanesthetized, and followed by cocaine administration through an indwelling brain cannula. Both low- and high-dose cocaine administration was followed by tachypnea; panting increased with tachypnea also accompanied high-dose injection. Due to the ACE the cocainized animals in respiratory patterns similar to those observed following intravenous and intraventricular administration described earlier. We suggest that the ACE projections to phase-switching areas in the pons or the projections to the nucleus of the solitary tract may denote a portion of the respiratory pattern changes following cocaine administration. Supported by ROI-DAO49173.

DIFFERENTIAL RESPONSE TO CYANIDE AND HYPOXIA OF ADULT CAROTID BODY GLOMUS CELLS. D.F. Donnelly, T.R. Cumes and C.S. Hedley. Dept. of Pediatrics, Yale Univ. School of Medicine, New Haven, CT 06510.

Both cyanide and hypoxia stimulate carotid chemoreceptors. In vivo and in vitro, but the mechanism(s) of this stimulation is obscure. To investigate the transduction process, we examined the membrane currents of glomus cells which were acutely dissociated from adult rabbit carotid bodies. Glomus cells were identified by their morphologic appearance, binding of FITC-labeled antinicotinic receptor antibody and ability to generate action potentials. Cells were voltage-clamped in HEPES buffer and recorded using a whole-cell patch technique. During the experimental period, the cells were stepped from -70 mV to 0 mV every 6 sec. Histochemical data indicated that stimulation was induced by brief applications (5-20 sec) of NaN (2-6 mM) in HEPES buffer. Hypoxic hypoxia was induced by injection of HEPES buffer equilibrated with nitrogen (PO2 10 mmlg). Cyanide caused a significant increase in late outward current (139±4 of the control, mean±SE, n=12) which occurred in all cells studied. Hypoxic hypoxia caused a significant decrease in the late outward current (mean±SE, n=9). Neither agent affected voltage-dependent inward current. These results show that histotoxic hypoxia and hypoxic hypoxia cause changes in the voltage-dependent outward current of glomus cells and suggest a fundamental separation of transduction processes for the two stimulatory agents.

ALTERATIONS OF TOTAL LUNG RESISTANCE (TLR) EVOKED FROM CAUDAL VENTROLATERAL MEDULLA (CVL) AND ROSTRAL VENTROLATERAL MEDULLA (RVL) IN DOGS. J.R. Harlow, P.A. Padgett, and M.P. Kaufman. Dept. of Cardiovascular Medicine, Univ. California, Davis, CA 95616

A previous report from this laboratory demonstrated that chemical stimulation of the solitary tract, in the dog, resulted in a reduction of TLR due to the withdrawal of cholinergic bronchodilator tone (J. Appl. Physiol. 63:92-97). The RVL was not examined in that study. In the present study, triple-barreled-type catheters were used to evoke responses. Each barrel was filled with one of the following: 100 mM DL-homocysteic acid at pH 3.6, 0.9% NaCl, or 2% Fast Blue. All solutions were adjusted to a pH of 7.40 using HCl or NaOH. Microinjections (25 nl) were made in the ventrolateral medulla at levels ranging from the obex to the caudal pole of the facial nucleus bilaterally following a pilot experiment.

In CVL, but not saline, elicited a decrease in TLR (8.2±0.7 to 6.7±0.6 cmH2O/l/min) and a reduction in blood pressure (BP; 133±4 to 111±5 mm Hg) at 5 sites. One site, in the nucleus ambiguus, evoked an increase in TLR (8.6±1.8 to 12.8±6.7 cmH2O/l/min) in response to BP (104 to 66 mm Hg) and a profound decrease to barocardiectomy (104 to 2 bpm). In RVL, but not saline, produced: a) an increase in TLR (6.4±0.5 to 6.7±1.2 cmH2O/l/min) and BP (135±9 to 165±14 mm Hg) at 9 sites; b) a reduction in TLR (10.5±2.0 to 8.6±0.5 cmH2O/l/min) and increase in BP (138±4 to 161±6.5 mm Hg) at 3 sites, or c) an increase in BP (125±11 to 145±15 mm Hg) without a change in TLR at 7 sites. The RVL sites, from which an increase in TLR was elicited, were consistently placed ventral to the nucleus ambiguous. Prossor sites which did not elicit a change in TLR as well as those which evoked a decrease in TLR were located either lateral (n=7) or posterior (n=1) to those which evoked an increase in TLR. The increase in TLR elicited from RVL was not altered following propranolol (1.5 mg/kg; i.v.) but was abolished by atropine methyl nitrate (1 mg/kg; i.v.). We conclude that CVL inhibits, while RVL activates, cholinergic drive to the airways.


The maintenance of a stable environment in the middle ear air space is critical for efficient transduction of airborne sounds to the fluid-filled inner ear and organ of Corti. In most mammals (including humans) auditory performance is critically related to individual fitness. As such, evolutionary processes must have been operating under strong selective pressures, especially with the advent of speech in modern humans where auditory-visual communication became the primary interactive medium. The neural mechanisms underlying homoeostasis of middle ear gas (air) composition and pressures are only just starting to be understood. We have demonstrated neural connections between the middle ear and the brainstem which may mediate such a mechanism. Although the circuitry has not been identified, the middle ear and auditory-visual modalities remain unknown. Our work has focussed on a population of cells, the gomus/ganglion cell clusters of the middle ear tympanic plexus, which show some similarities to those of the carotid body/sinus, and may represent an analogous sensory apparatus. We have suggested that the late appearance of these cells during primate development is related to the extremely high incidence of otitis media in humans. In the present study we saw degenerating axosomatic synapses on gomus cells, bilaterally, after unilateral SCCectomy. The cell clusters, comprising approximately 100-150 cells are thus associated with both sensory and autonomic elements. Ultrastructural analysis did not reveal features which characterize carotid system sensory elements, such as large dense cored vesicles and fenestrated endothelia. However, their function as chemotopo-receptors could not be ruled out. For example, the clusters were often associated with an extensive and tortuous vascular bed, suggesting a (middle ear) blood gas pressure sampling system with perhaps different physiologic characteristics than the carotid system.

ROLE OF CAPSAICIN-SENSITIVE BARO- AND CHEMOAFFERENTS IN THE CONTROL OF BLOOD PRESSURE AND RESPIRATION. G. Daniell and T.R. LaHann. College of Pharmacy, Idaho State University, Pocatello, ID 83209.

Although capsaicin inactivates small diameter sensory neurons, most reports suggest that capsaicin pretreatment has little, if any, effect on blood pressure or respiration. This implies that unmyelinated afferents are unlikely to be important in control of respiratory or cardiovascular function. Our results do not entirely support this interpretation. We have investigated the effect of local application of capsaicin and chemosensitive afferent function. Capsaicin or its vehicle was applied to the vagus, carotid sinus and glossopharyngeal nerves. Baroreflex function was evaluated 2 hours post treatment in unanesthetized rats and 24 hours post treatment in conscious, freely moving rats. Neither capsaicin nor its vehicle altered the relationship between pressure responses and bradycardia. In anesthetized rats, capsaicin pretreatment altered the nature of the baroreflex response without attenuating it. Local application of capsaicin to anesthetized rats also blocked the tachypneic response to stimulation of carotid chemoreceptors (e.g., CO₂, capsaicin) and eliminated chemoreceptor-induced pressor responses. We interpret these results to mean that capsaicin-sensitive chemoreceptors may be important in the control of respiratory function, but that capsaicin-sensitive baroaffereents are unlikely to modulate baroreflex function in conscious animals under low stress conditions.
436.17 TRIGEMINAL INJECTIONS OF LIDOCAINE INHIBIT THE CARDIORESPIRATORY RESPONSES TO STIMULATION OF THE SUPERIOR LARYNGEAL NERVE IN THE MUSRATKAT. W.M. Panettore, Dept. of Anat. & Neurobiology, St. Louis University Med. Sch. of Med., St. Louis, MO 63104. Electrical stimulation of the superior laryngeal nerve (SLN) induces apnea and bradycardia, similar to the responses seen after stimulation of the nasal cavity. In order to determine the continuing role of the trigeminal fibers which project to both the nucleus tractus solitarii and the caudal trigeminal complex (MDH). The purpose of this study was to determine if blocking the trigeminal projections affects the cardiorespiratory responses to SLN stimulation.

Muskrats were anesthetized, their SLN isolated bilaterally, and placed on headstage St. Louis University Med. Sch. of Med., St. Louis, MO 63104. The nasopharyngeal pressure was monitored on a physiograph. Bilateral injections (100-200ng) of 2% lidoceaine were placed in the MDH and the SLN stimulated (0.5-4mA; 10-40Hz/2s). When the SLN injections were placed in ventral parts of the rostral MDH, especially those centered in laminae I and II, the apnea and bradycardia usually elicited by SLN stimulation were reversibly inhibited. These data suggest trigeminal neurons are important for the cardiorespiratory responses after SLN stimulation. Moreover, the rostral MDH may modulate the autonomic responses to stimulation of the whole upper respiratory tract since similar injections inhibit the responses seen after nasal stimulation. Supported by NIH grant HL 38471.

REGULATION OF AUTONOMIC FUNCTION: CONTROL OF LUMBOSACRAL AUTONOMIC OUTFLOW

437.1 LIMIT CYCLE AND NEGATIVE FEEDBACK IN BLADDER CONTROL FOLLOWING SPINAL CORD INJURY. J.S. Walter, P. Zgierszynski*, J.S. Wheeler*, R.D. Wurster, Rehab. R&D Center, Hines VA Hosp., Hines, IL 60141. We have begun to explore models of a bladder control system following spinal cord injury by conducting perturbation analysis and nerve anesthesia/stimulation studies. Periodic bladder contractions occurred in chronic, unanechastised, spinal-injured, male cats (T-1) when the bladder volume was maintained above the micturition threshold, 30 to 60 ml. A perturbing volume of 3 to 5 ml phase advanced or delayed the bladder contraction depending on whether the volume was added or withdrawn respectively. The period, however, was unchanged. These observations are typical for rhythms controlled by a stable limit cycle system.

In addition to the known positive feedback for bladder control, the presence of negative feedback was indicated in the anesthetized animal by comparing declines in bladder contractions when the pelvic nerves were intact and after direct bladder stimulation with crushed pelvic nerves. In both cases declines in pressure had similar slopes. Therefore, with intact pelvic nerves, declines in bladder pressure during spontaneous bladder contractions may be due to inhibition of spinal cord "turning off" pelvic motor activity, analogous to turning off our direct bladder stimulator. Supported by Rehab. R&D Center and Merit Review Grant B441.


The possible sites of action and effects of KM-801 (1mg/kg; 10mg/kg) in the bladder of the rat via a transurethral catheter were compared in five preparations: 1) Intact rats 2) Chronic spinal (76 - 78 rats) 3) Unanaesthetised, preganglionic rats 4) Intact rats treated 17-24 hours following reserpine (5mg/kg I.M.) 5) Intact rats with KM-801 injected intrathecal (I.T.) catheter at the L6-S1 spinal cord segment. Animals were examined with urethan (2.5mg/kg S.C.) except the decaerbeated animals, whose surgery and preocollinales deacrerebration was performed under halothane (2% anaesthesia). KM-801 (0.3-3mg/kg I.V.) given to intact rats produced complete inhibition of bladder activity. In deacrebrate and chronic spinal animals the same and considerably higher doses (3-10mg/kg I.V.) little or no effect on the bladder activity. Intrathecal administration of KM-801 to intact rats inhibited bladder contractions at a total dose of 12-36 mg. Mean pressure prevention of the inhibition produced by KM-801 in 50% of the animals tested. These data suggest that at least 2 possible sites of action of KM-801: the forebrain rostral to the superior colliculus and the spinal cord. The latter may depend upon descending input from the forebrain. The reduction of KM-801 effects in desparaeradized rats suggests that in addition to an action on central nervous system (CNS) acid synapses that there is also an involvement of catecholamine and/or 5-HT mechanisms in the action of KM-801.

437.3 ROLE OF CHP AND SHT IN CENTRAL PATHWAYS CONTROLLING MICTURITION IN THE RAT. T. Suzuki, M. Kawatani, S. Redman, M.C. deGroat. Dept. of Pharmacology and Behavioral Neurosciences, University of Pittsburgh, Pittsburgh, PA 15261.

Bulbospinal pathways from the pontine micturition center (PMC) and raphe nuclei to the parasympathetic nucleus in the lumbar sacral spinal cord of the rat contain CHP and SHT, respectively, and have been implicated in the central control of micturition. The present study examined the role of these pathways in regulating bladder reflexes (BR) in urethane-anesthetized rats. In normal rats, distension of the urinary bladder (UB) evoked rhythmic bladder contractions and firing on UB postgonadil nerves. BR were acutely blocked by bilateral electrolysis lesions (2mA;10sec) in the PMC but recorded 7 days after the lesion. Ablation of the spinal cord (I. L segment) of CHP (10.3-30pg) depres transiently of 5 days prior to experiments with 5-HT receptor antagonist 100mg/kg (SP). These findings indicate that 5-HT neurons in the lumbar sacral segment have facilitatory and inhibitory influences, respectively, on micturition. Since 12 CHP inhibits UB activity it seems likely that this peptide is not the transmitter involved in the descending excitatory pathway from the PMC to the lumbosacral parasympathetic nucleus.

437.4 HOMOGENIZATION IN CAT VISCERAL GANGLIA FOLLOWING SACRAL ROOT SECTION. A.M. Booth, M. Kawatani, B. Sheerik, M.C. de Groat. Deps. of Pharmacol. & Behav. Neurosci. Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

The sacral parasympathetic preganglionic input to the urinary bladder leads to: 1) abnormal sympathetic stimulation of the bladder (e.g. hyporesponsiveness of spontaneous and stimulus evoked activity in bladder ganglia (BG) and 2) to the induction of a series of reflexes (e.g. ureteric propulsions P (SP)). These findings illustrate the plasticity in BG following chronic partial decentralization. This study utilized acute and immunoradiometric recordings to examine decentralized BG (DBG) in vivo and in situ 15 weeks to 15 months after lesioning in 5 rats in 3-100 cells in BG of cells exhibiting synaptic responses to electrical stimulation (ES) of preganglionic nerves (PGN) was smaller (25-65%) than in normal BG (85-100%) while the % of cells in DBG exhibiting spontaneous firing was much higher (25-55% vs 0-5%). Mean firing rate in the BG of DBG was unchanged (20-40 mV) "spontaneous" epsps. Repetitive ES (2-50Hz) of PGN elicited an increase in spontaneous activity that was positively correlated with the amplitude and duration of the ES. Cells in DBG received fewer inputs (2-4 vs 6-7) and while the BG was unchanged (8-15 mV) the probability of eliciting firing was greatly increased. Both SP and VIP evoked asynchronous firing at doses that had no effect in the BG. In summary we conclude that sympathetic plasticity in DBG may markedly change the efferent input to the bladder and may contribute to the development of the autonomous hyperactive bladder.

(Supported in part by NS-22436, NS-25254 & AM-37241)
437.5
BLADDER CONTRACTIONS EVOKED BY MICROSURGICAL STIMULATION OF THE SACRAL SPINAL CORD J.R. Roppolo, A.M. Booth, S. Smerin, L. Nadelhaft, W.C. de Groat, and W.C. de Groat. Dept. Pharmacology, Univ. Pittsburgh School of Medicine, Pittsburgh, PA 15261
This study examined the effect of focal electrical microstimulation of the sacral spinal cord on bladder activity. In intact adult male chloralose anesthetized cats, the bladder was cannulated for pressure recording and a laminectomy exposed the lumbar sacral spinal cord (L-7 and 1) and roots. The sacral segment containing bladder dysfunction was identified by stimulation of each ventral root (10-15 Hz, 0.05 sec pulse). S root stimulation produced maximal responses (60-100 cm H2O). The S, C, and T roots produced small (1-5 sec duration, 0.2 m sec charged pulses, 5-25 uA) with a tungsten microelectrode. A large number of dorsal root afferents were identified toward the PGN of the sacral parasympathetic nucleus (SPN). Bladder contractions (maximum amplitude of 20-40 cm H2O) were evoked over a distance of 0.5-0.8 mm in the intermediolateral gray (1.5-2.3 mm from surface) and were not usually associated with great somatic motor activity. Stimulation dorsal or ventral to the SPN could evoke bladder contractions, but thresholds were higher and skeletal muscle responses prominent. Bladder contractions were abolished ganglion blockade (trimethaphan 0.25 mg/kg) but were unchanged following neuromuscular blockade (pancuronium 0.1 ng/kg). These studies demonstrate that bladder contractions can be evoked selectively by focal electrical stimulation of the sacral spinal cord. (Supported by NIH contract ROI-NS-9-2366)

437.6
This study utilized the fetal and neonatal rat bladder with the following observations. The bladder to bladder reflex was lost by day 6. It has demonstrated that the absence of the bladder-to-bladder reflex at birth is due to the degeneration of the sensory afferents from the bladder. This study addressed the question of whether the neonatal rat is born with an intact sensory pathway from the bladder to the spinal cord. Bladders were exposed toward the neonatal rat. The spinal cord was and dorsal root ganglia (DRG) were removed 10-48 hours later for TMB processing. WGA-HRP labelled cells were seen in the L5 DRG (>75%) and S, DRG (>75%) in day 0 to day 9 pups. WGA-HRP labelling was also seen in the sacral parasympathetic nucleus and lateral dorsal horn of the 4, 5, 6, and 7 spinal cord of day 0 through day 9 pups. These data indicate that bladder afferent neurons at birth have a segmental distribution and central projections similar to that of adults. The lack of a functional bladder-to-bladder reflex before postnatal day 6 could be due to immaturity of different spinal mechanisms or interneuronal pathways in the CNS.

437.7
NEUROBIOTIN (NB) AND HRP DEFINED DENDRITIC MORPHOLOGY SUGGESTS ADDITIONAL FUNCTIONs FOR SACRAL PREGANGLIONIC NEURONS (PGN) R.J. Brand* , J.R. Roppolo, D.L. Saltiel, W.C. de Groat, and W. Nadelhaft. Dept. Anatomy and Neurobiology, University of Virginia Medical School, Norfolk, VA 23501; Dept. Pharmacology, Univ. Pittsburgh, Pittsburgh, PA 15261
PGN in the lateral band (LB) of the sacral parasympathetic nucleus of the male cat are presumed to subservie various urogenital functions. Intracellular labelling was performed on more than 70 LB neurons with B- fiber axons to determine whether different subpopulations could be distinguished morphologically or functionally. 1. Neurons were identified based on combinations of dendritic distributions in (1) the lateral funicularis, (2) lamina II, (3) the dorsal commissure and (4) the ventral horn. Groups of cells had dendritic projections to regions: 1-2, 3-4 & 3-4; 2-3 & 4; 3-4 & 4 or 3-4. 2. Data suggest that the functions of sacral lateral band PGN (bladder, penile, urethral, seminal vesicles) must be correlated with and predicted by their dendritic patterns. Further studies are underway to directly test this hypothesis by identifying the function of cells prior to intracellular labelling. Supported by NINDS RO1 NS26588

437.8
NEURONS LABELLED FOLLOWING THE APPLICATION OF TRACERS TO THE DIGITAL CUT HYPOGASTRIC NERVE OF THE RAT: L. Nadelhaft and P. L. Vera. VA Med Ctr & Dept of Neuroscience and Pharmacology and Center for Neurosci, Univ of Pittsburgh, Pittsburgh, PA 15261
Following the work of Brown and Janig [JAN 24:401'88] in the cat, we applied dyes to the distal stump of the transected hypogastric nerves (HGN) of the female rat. The animal was anesthetized with halothane and one hypogastric nerve dipped into fast blue and the other into fluorescein. After 10 days, the animal was reanesthetized with pentobarbital and perfused with 4% buffered paraformaldehyde. Labelled neurons numbering in the hundreds were located mainly ipsilaterally; however there were significant numbers of contralateral cells present. In the major pelvic ganglion (MPG) cells were located in the T13-L3 (35%) and L6-S1 (65%) dorsal root ganglia. In the sympathetic chain, labelled neurons were found between T12 and S2. A few preganglionic neurons were found in spinal cord segments L6 and S1. These groups of neurons may be innervating portions of the reproductive system served by small nerve branches leaving the HGN between the inferior mesenteric ganglia and the MPG.

437.9
SYMPATHETIC POST-GANGLIONIC INNERRATION OF THE RAT URINARY BLADDER. Predis L. Vera and Irving Nadelhaft. VA Med Ctr Ctr & Dept of Pharmacology and Neurosurgery, University of Pittsburgh, Pittsburgh, PA 15240.
Female rats were anesthetized with halothane and the urinary bladders exposed. Fluorescent tracers (Fast Blue or Fluoro-Gold) were injected into the bladder wall using a Hamilton syringe. One to 3 weeks after the injections, the rats were anesthetized and perfused with phosphate buffered saline. Major pelvic ganglia (MPG), the inferior mesenteric ganglion (IMG) and the sympathetic chain (T10-S3) were removed, sectioned (20 um) in a cryostat and examined for the presence of retrogradely labeled cells. The sections from the MPG were also processed for DNH-immunohistochemistry. In addition, retrogradely labeled cells were found in the IMG. In the MPG, many retrogradely labeled cells were present and some also contained DNH immunoreactivity. In addition, retrogradely labeled cells were found in the IMG. In the MPG, many retrogradely labeled cells were present and some also contained DNH immunoreactivity. In addition, retrogradely labeled cells were found in the IMG. In the MPG, many retrogradely labeled cells were present and some also contained DNH immunoreactivity. 

437.10
The afferent and efferent pathways to the cat eschatoavenous muscle (ICM) were identified using the retrograde marker WGA-HRP, and cholora toxin B-HRP injected into the ICM under halothane anesthesia. 72 to 96 hours later anesthetized cats were perfused with fixative. Spinal cord and dorsal root ganglia (DRG) from L3 to S2 were processed with TMB to reveal HRP. Lower motoneurons (average, 150) projecting to the ICM were present exclusively in Onuf's nucleus (ON), in L4, and S1. The neurons were typically elongate ranging from 250x to 400x microns in size. The neurons sometimes formed clusters, but were not confined to any particular division of ON. Chlorox toxin B-HRP revealed dendrites up to one millimeter long extending from the ventrolateral division of ON to the medullary ventral horn, the lateral funiculus, and the sacral parasympathetic nucleus. Labeled DRG cells (~1000 per gram in L3, S1) ranged from 10x20 to 80x110 microns in size. Sparse afferents extended through the medial and lateral collateral pathways into the dorsal funiculus. The distribution of the ICM overlaped with that of the striated urethral and anal sphincters, suggesting that coordination of the three may take place through the sacral spinal cord. (Supported by NIH contract ROI-NS9-2366)
437.11 INHERITANCE AND FUNCTION OF THE ISCHIOURETHRAL MUSCLE OF THE RAT. N. G. Dall, J. G. Walsh, K. E. McKenna, Dept. of Anatomy, Univ. New Mexico Sch. of Med., Albuquerque, NM 87131 and the Dept. of Neurology, Yale University School of Medicine, New Haven, CT. 06020

The ischio urethralis, a striated perineal muscle presumed to be involved in sexual reflexes in the rat, is studied in the rat. The paired muscle arises from the pubic crest and attaches to the side of the bulb of the urethra. It has a central structure that is identified as the subpubic ligament in other descriptions. Retrograde staining studies show that the muscle is innervated by neurons in the dorsolateral nucleus of the lumbar spinal cord, a pudendal nerve motor nucleus which also projects to the ischiocavernosus muscle. Excision of the ischio urethralis muscle did not interfere with the ability of males to display normal copulatory behavior, nor did it affect significantly the number and intensity of reflexive erections. The function of this muscle and that of other perineal muscles remains to be determined.

437.12 A ROLE FOR 5-HYDROXYTRYPTAMINE IN MEDIATING SPINAL SEXUAL REFLEXES. L. Moran and K. E. McKenna Dept. of Physiology, Northwestern Medical School, Chicago, IL 60611

We previously identified a neurotransmitter in the veno-provocative perineal somatosensory afferents mediating the inhibition of spinal sexual reflexes. The present study provides neurochemical and pharmacological evidence for 5-hydroxytryptamine (5-HT) in the mediation of this inhibition. Rhodamine-labelled beads were pressure injected into LS-L6 of the spinal cord. After 7-14 days rats were perfused with 4% paraformaldehyde solution. The spinal cord and medulla were cut (350-400um) and processed for autoradiography using the immunofluorescent technique. Retrogradely labelled neurons were found in the PNU, raphe pallidus, raphe obscurus and gigantocellular reticular formation after injections located to the pudendal motor neurons. Many of these retrogradely labelled cells in the PNU contained 5-HT-immunoreactivity. Dense 5-HT-immunoreactive fibres and presynaptic terminals were found surrounding the perinodal motor neurons and interstitial spinal grey of the lumbar spinal cord. Male rats were anesthetized with urethane and an intrathecal catheter was inserted into the subarachnoid space, positioned with the cannula tip at L5-L6 of the spinal cord. The rats were spinalized. The coital reflex was elicited via a catheter inserted into the urethra. The reflex was monitored by ECG recordings of the bulbospongious muscle. Microinjection of 5-HT (1-2.5ug) resulted in an increased sensory threshold and delayed the coital reflex. Injection of higher doses of 5-HT (5-30ug) resulted in inhibition of the previously evoked coital reflex. Thus, 5-HT appears to alter the spinal pattern generator for the coitus reflex.

These findings provide evidence that 5-HT neurones in the rostral medulla mediate inhibition of spinal sexual reflexes.

437.13 MODULATION OF THE THRESHOLD FOR FICTIVE SEXUAL CLIMAX IN FEMALES BY PERIPHERAL SEROTONIN K.E. McKenna and K. E. Walsh* Dept. of Physiology, Northwestern Medical School, Chicago, IL 60611

We previously identified urethral stimuli as sufficient for eliciting fictive sexual climax in anesthetized, spinedale male rats [see McKenna, L. S. 1969, J. Sex. Res. 5: 23]. Simple mechanical stimuli alone are effective, some observations have led us to consider that urethral chemoreceptor mechanisms may play a role in eliciting fictive sexual climax. The present study was designed to identify the substructures of the urethral chemoreception.

Other investigators have shown in a number of species that the urethral mucosa contains serotonergic paracrine cells. The morphology of these cells suggests a chemoreceptor function. To determine if these paracrine cells are present in the rat, male and female rats were anesthetized and perfused. The urethra was removed and either 50 or 500 um transverse sections cut, or a wholemount preparation of the urethral mucosa was prepared. Immunofluorescence staining for serotonin revealed a large number of small (<10 um) heavily stained cells throughout the urethra. There was a concentration of these cells in the urethral bulbodistal urethra. This is the area which we have identified as most sensitive in eliciting fictive sexual climax. These cells have processes which extend to the luminal surface. In the submucosa, the cells were observed in two large clusters.

To examine the physiological role of these cells, female rats were anesthetized, spinedale and urethral catheter inserted via the bladder. Dimensions of the urethra by infusion of saline were followed by urethral mucosae occlusion in response to fictive sexual climax, as monitored by pudendal nerve recordings. The pressure threshold required to elicit the fictive sexual climax was highly consistent in a given animal. The addition of serotonin to the infusion fluid significantly and reversibly decreased (by 20-30%) the pressure required to elicit the response. The maximal effective dose was 10^-6 M.

437.14 MAINTENANCE OF ERECTION OF PENILE GLANS, BUT NOT PENILE BODY, AFTER TRANSECTION OF RAT CAVERNOUS NERVES. B.D. Sachs and K. E. Walsh, Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020

A role for the cavernous nerve (CN) in erection of the corpora cavernosa has been well documented. Evidence for CN-innervation of the corpus spongiosum implies a role in erection of the glans penis. In this study, the role of the CN on erection of the penile body, not the glans (Quinlan et al., J. Physiol., 156: 551, 1989). To further examine the role of the CN in glans erection, we tested male rats for mating behavior and sexual reflexes after CN transection (CNx). Male rats were trained to display weak and moderate glans erections, but lacked penile body erections and intense glans erections. These results suggest that at least in rats the innervation of the corpus cavernosum differs from that of the corpus spongiosum, and that the CN may play little or no role in corpus spongiosum function.

[Sponsored by NIH research grant HL-08933.]

437.15 NORMAL SEXUAL REFLEXES IN THE ABSENCE OF URETHRAL STIMULATION IN INTACT COPULATING RATS. G.M. Holmes, D.B. Lynch* and R.D. Sachs, Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269

The absence of seminal and prostatic fluids does not alter the motor patterns or parameters of copulation, including the ejaculatory response (ER), in rats (e.g., Tisell & Larsson, Invest. Urol. 10: 374, 1979). Research on anesthetized spinalized rats suggested a role for urethral stimulation in the ER, as measured by the EMG of the bulbospongious muscle [Matt, Chung et al., Neurosci. Lett. 34: 40, 1989]. In order to help place this finding in a natural setting, we studied intact rats as after penile insertion and continuing for several seconds after coitus. Anesthesia of the urethral mucosa with lidocaine did not disrupt this EMG response of the bulbospongious muscle. Injection of glutamate into the bulbospongious plunger had no effect on the ER. These results suggest that urethral stimulation by the ejaculatory reflex is not dependent on the stimulation of the striated muscle component of the ejaculatory related reflexes in copulating male rats.

[Sponsored by NIH research grant HD-08933.]

437.16 FUNCTIONAL PLASTICITY IN DECENTRALIZED AUTONOMIC GANGLIA: LACK OF EVIDENCE FOR MAST CELL INVOLVEMENT. N. Minorsky, G. Wiltznt and M. G. Dall, Dept. of Anatomy, Univ. New Mexico Sch. of Medicine, Albuquerque, NM 87113

Stimulation of the hypogastic nerve in the rat results in penile vasodilation when the pelvic nerve is interrupted. The mechanism for this response is unknown but in view of the hyperneuritic status of denervated ganglion cells. No recent suggestion that mast cell products increase excitability of autonomic ganglion cells. We have performed ultrastructural analysis of mast cells in intact and partially denervated major pelvic ganglia (MPG). The number of mast cells in the MPG was determined from light and electron microscopy studies. The average number of mast cells 1 S.E.M./MPG was unchanged when intact ganglia were compared to ganglia in which the pelvic nerve was interrupted seven days after nerve transection. No difference was seen between control and denervated ganglia when an arbitrary scale was used to assess mast cell degeneration. Although it remains possible that mast cells could alter ganglionic transmission following injury, the present study does not change in mast cell number and activity coincident with functional plasticity.
437.18 URINE RETENTION DUE TO INTRASPINAL INJECTION OF CO-CHLORCIDE: THERAPEUTIC EFFECT OF BEMACIN, NERVE GROWTH FACTOR AND NONOSILOLANGLOSIDO GLICONE. H. Fardal, E. Greenfield, J. Grunberg, M. Truzzi*. Chair of Pharmacology and Pharmacogeny, School of Pharmacy, Modena University, 41100 Modena, Italy. We described that the intraspinalse injection of low doses (2-5 ug/rat) of colchicine (C) induces a massive urine retention, bladder hypertrophy and urine overflow. Pharmacologic agents that are typically central to the neuronal circuitry underlying bladder dysfunction has not been studied in the rat. We have shown that Propranolol did not trigger bladder voiding. Neuropeptides have been show to play a key role in the regulation of the micturition reflex in the spinal cord and bladder function. In rats with C-induced urine retention we found at the site of injection an increase of Met-enkephalin, a marked decrease of Substance P and no apparent change at the level of Dynorphin A. The urine retention was worsened by Naloxone whereas was prevented by Brexamoline, a K and sigma receptor agonist. In the spinolateral cord of rats with urine retention we detected an increased presence of K and sigma receptors. It seems likely that Brexamoline, despite its K stimulatory activity, by stimulating sigma receptors present on the Mg-sensitive NMDA-glutamate receptors, allosterically inhibits an increased NMDA receptor activity. An implication of a glutamatergic dysfunction in the C-induced urine retention seems to be confirmed by the ability of Nerve Growth Factor and Nonosilolangloside (GnW), which minimise the neurotoxicity of glutamate, to normalise urine output in C-treated rats.

NEUROENDOCRINE REGULATION: OXYTOCIN, VASOPRESSIN

438.1 PROPERTIES OF POSTERIOR PITUITARY PRIMARY CULTURES. C. Dora* , A.K. Salam, R.B. Meeker, L. Ritz*, R.S. Greenwood and J.N. Greenwood, Department of Neurology, Pediatrics, Pharmacology and Neuroscience Carriier, University of North Carolina, Chapel Hill, NC 27599. Pituitaries were cultured from the neural lobe of the rat pituitary at developmental ages E16-PN14 and adults. Cultures were grown from both punches of the isolated neural lobe and dissociated cells. The earliest age at which cultures were effectively grown was E17-P16. Cells from E18 fetuses exhibited process-bearing and flat-polgonal morphologies, similar but not identical to cultured astroglia. The process-bearing cells exhibited strong GAP immunoactivity whereas the flat-polgonal cells had variable levels of staining ranging from robust to weak. As the neural lobe developed, fewer process-bearing cells were observed in culture, until by PNS-1, cultures were largely comprised of flat-polgonal cells and a few small flat cells with or without irregular processes. Cultures of neural lobe punches at PNS-1 contained a core of flat cells surrounded by spindly-shaped, fibroblast-like cells whereas, dissociated cells yielded more homogenous cultures that were 70-90% GAP immunoreactive. A majority of the cells also stained robustly for fibronectin. A subpopulation of cells from cultured neural lobe found to express specific vasopressin binding sites. Stimulation with 10^{-7} M vasopressin, oxytocin and norcynephrine resulted in increased intracellular calcium in subsets of flat-polgonal unidentified cells. Oxytocin and leu-enkephalin had no effect on intracellular calcium concentration. Supported by NIH Javits Award NS 13411.

438.2 PRIMARY CULTURES OF MAGNOCELLULAR NEUROENDOCRINE CELLS FOR IN VITRO PATCH CLAMP STUDIES AND ANATOMICAL ANALYSIS. L. Ogilvie, J. Ritz*, E. Cox*, R.B. Meeker, R.S. Greenwood and J.N. Greenwood, Dept. of Neurology and Dept. of Pathology, University of North Carolina, Chapel Hill, NC 27599. To identify optimal culture conditions for physiological studies of magnocellular neuroendocrine cells, the properties of fetal and early postnatal, anterior hypothalamic cultures containing supraoptic and paraventricular neurons, were evaluated under different conditions of tissue preparation and culture. Tissue punches through the region of the supraoptic nuclei, coronal slices or dissociated cells were cultured on coverslips coated with either polyl-lyine, collagen (Vitrogen) or sublimated tissue basement membrane (Matrigel). The growth characteristics of magnocellular neurons differed under various conditions. Slices and tissue punches produced a mix of healthy neurons which slowly migrated short distances from the cell mass. The migration and axonal outgrowth appeared to be limited to the region of glial outgrowth from the mass. In contrast, a collagen matrix supported rapid axonal outgrowth from cultures which extended past the glial outgrowth. This latter type of preparation appears to be best for axonal outgrowth but was less useful for patch clamp studies. Large cells with a morphology similar to immuno- cytochemically identified magnocellular neuroendocrine cells were easily identified in dissociated cultures. These cells could be patch clamped and infused with Lucifer Yellow. These cultures provide a means by which vasopressin and oxytocin magnocellular neurons can be cultured for different growth properties, studied using standard patch clamp methods, marked intracellularly with Lucifer Yellow, and identified immuno-cytochemically. Supported by NIH Javits Award NS 13411.

438.4 RAPID VASOPRESSIN mRNA EXPRESSION IN THE RAT HYPOTHALAMUS DURING POLYETHYLENE GLYCOL-INDUCED HYPOVOLEMIA. R.B. Meeker, E. Perkins*, M.M. Nicolle, L. Ritz*, R.S. Greenwood and J.N. Greenwood, Dept. of Neurology and Pediatrics and Neurobiology Curriculum, University of North Carolina, Chapel Hill, NC 27599. The study of the control of vasopressin mRNA expression in magnocellular neurons is hampered by the slow rate of blood volume or osmolality changes following most experimental manipulations, such as water deprivation or hypovolemic saline injection, which may be rate limiting. Consequently, we characterized the increase in VP mRNA levels by a single subcutaneous injection of polymerized glycol (PEG) and the time course of VP mRNA increases in the supraoptic and paraventricular nuclei in a response to a more rapid challenge. A 30ml oligonucleotide probe specific for vasopressin mRNA was hybridized to 20 um sections of rat brains 0, 4, 8 or 16 hrs after PEG injection or 24 hrs after injection in rats which were allowed to regulate their water intake. Let in rats which received VP mRNA increased within 4 hrs of PEG injection to values approximately, 2-fold greater than control values. The similarity of this time course to the evoke release of vasopressin suggests that the two processes may be under similar transsynaptic control. This paradigm may provide a simple and useful model for in vivo pharmacological analysis of pathways which control vasopressin gene transcription. Supported by NIH Javits Award NS 13411.
OSMOTICALLY STIMULATED AVP SECRETION IN RATS. Eric A. Blackmon, M.D., and Alan G. Robinson, M.D. Departments of Neurosurgery and Medicine, University of Pittsburgh, Pittsburgh, PA 15261. Opioids are reported to act directly on the posterior pituitary to inhibit release of neurohypophyseal peptides, but this has been difficult to study with intravenously administered drugs in intact animals because of the multiple potential sites of action of opioids throughout the central nervous system. We developed a technique using stereotactic surgery, local anesthesia and retrograde infusion of morphine into the posterior pituitary to directly infuse substances into the matrix of the neurohypophysis. After cannulation under anesthesia with equilibrating basilar vasopressin levels in plasma were 10.8 ± 2.3 pg/ml. When a 5 μl solution of 1.6 mM potassium chloride was infused slowly into the substance of the gland, plasma vasopressin peaked at 10 ± ± 6 pg/ml, and was significantly above pre-infusion levels 120 min after the injection of a 5 μl solution of 5 μg morphine in the same 5 μl solution, vasopressin release was inhibited with a value of 3.3 ± 0 pg/ml at 5 minutes, a slight rise at 15 minutes and return to basal. Injection of morphine peripherally at the same concentration had no effect on the release of vasopressin during high potassium stimulation. Thus, we have used an in vivo technique to document that opioids delivered to the neurohypophysis directly inhibit the release of vasopressin independent of action elsewhere in the central nervous system.

TOPOGRAPHY OF HYPOTHALAMIC OXYTOCIN NEURONS THAT EXHIBIT c-fos IMMUNOREACTIVITY UPON OSMOTIC STRESS IN THE RAT. Lisa Giovannelli, Privatiss. J. Shionami, Gustav F. Jirikowski and Floyd E. Bloom, Dept. Neuropharmacology, Scripps Clinic Research Foundation, La Jolla CA 92037 and Dept. Psychiatry, UCSD, La Jolla CA 92039. In order to evaluate the metabolic responses of specific neurons to osmotic perturbations in vivo, we have visualized c-fos and oxytocin (OXY) expression in rat brain by double-immunostaining. Both a monoclonal and a polyclonal antibody raised against c-fos peptide with sequence 4-17 were used. In normal untreated male rats, or in male rats treated with intraperitoneal injection of isotonic saline, no OXY-immunoreactivity was expressed detectable c-fos immunoreactivity. However, nuclear c-fos immunoreactivity was readily detectable in OXY neurons at this time point. Immunoreactive neurons for both OXY and c-fos were found in the supraoptic nucleus, in the paraventricular nucleus and in the lateral subcommissural nucleus. These results suggest that oxytocin neurons are more metabolically activated by osmotic stimuli, and indicate the need to further understand the role of the neurons expressing this neuropeptide. Grant n. NS22347.

ANTERIOR HYPOTHALAMIC (AVX) LESIONS IMPAIR MAXIMAL SUPPRESSION OF VASOPRESSIN SECRETION IN RATS. R.E. Blackmon, E.M. Stricker and J.G. Vertbals, Departments of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15261. Lesions of the anteroventral third ventricular (AVX) region severely impair osmotically-stimulated secretion of vasopressin (AVP) and oxytocin (OXT) from the rat neurohypophysis. Differential neurophysiological mechanisms may underlie these responses, an osmoreceptor-mediated process, we studied the ability of AVX-lesioned rats to excrete free water loads as an index of their AVP suppressibility. Radiofrequency lesions were made in male rats (2 mm probe with 2 mm tip to exposure; 7.3 mm below bregma in the midline), and the adequacy of the lesion was evaluated subsequently by the absence of drinking 1 hr after intraperitoneal injection of a hyperoncotic (1.5 M NaCl) salt solution. At this time point neurons immunoreactive for both OXY and c-fos were found in the supraoptic nucleus, in the paraventricular nucleus and in the lateral subcommissural nucleus. These results suggest that oxytocin neurons are more metabolically activated by osmotic stimuli, and indicate the need to further understand the role of the neurons expressing this neuropeptide. Grant n. NS22347.

FOS EXPRESSION DEMONSTRATES HETEROGENEITY OF MAGNOCELLULAR VASOPRESSIN AND OXYTOCIN RESPONSE. M.M. Roberts, A.G. Robinson, M. Finkenrumpf, and J.C. Hoffman, Departments of Medicine, University of Pittsburgh, Pittsburgh, PA 15261. Activated vasopressin (AVP) and oxytocin (OXT) neurons of the supraoptic (SON) and paraventricular (PVN) nuclei are involved in the osmoregulatory and neuroendocrine system, respectively. To help understand the processes involved in transcription, c-fos expression was used to map the response of individual magnocellular neurons to graded hemorrhage in rats. Following a 2 cc bleed, 12% of AVP neurons responded, while 2% of OXT neurons expressed c-fos. In the retrograde infused rats these neurons expressing fos, 26% expressed fos at a low intensity level and only 4% expressed fos at the highest intensity level. After a 6 cc bleed, 52% of AVP neurons expressed fos compared to 10% at low intensity and 38% at the highest intensity. As the degree of stimulus increased, both the total number of cells responding fos, and the intensity of fos expression increased. For expression in cells double-labeled for AVP or OT delineated hormone-specific differential functional anatomy in the SON and PVN. In the SON, after 6 cc hemorrhage, 19% of AVP neurons, but only 2% of OT neurons were activated. In the PVN, there was no activation of AVP neurons, but 26% of OT neurons were activated. A similar differential response pattern was seen with 4 cc hemorrhage, but with 6 cc most neurons were activated in both nuclei. Fos Immunoreactivity thus provides a permanent record of activated neurons in the SON and PVN. Dual immunostaining for fos and AVP or OXT was used to demonstrate a previously unrecognized functional anatomic heterogeneity in the threshold for response of magnocellular neurons to hemodynamic stimuli.

WATER-DEPRIVATION INCREASES GLUTAMINE UTILIZATION IN THE HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM AND CIRCUMVASCULAR ORGANS. M. Kadekar, S. Freeman, M. L. Terrell and J. S. Harris, Division of Neurosurgery, University of Texas Medical Branch, Galveston, Texas 77550. We studied with the quantitative [(14C)l-glutamate autoradiography the neural pathways activated by 24, 48 and 72 hr water-deprivation and intravenous infusion of hypertonic saline (2.5 M, 250 μl/min for 4 min) in adult male Sprague-Dawley rats (n=61). Water-deprivation over 7 hr induced a progressive increase in plasma osmolality and hemocrit. Hypertonic saline increased plasma osmolality to a larger degree than 72 hr water-deprivation (a:27 ± 16 mOsm/kg). Hypertonic saline increased glucose utilization in the supraoptic and paraventricular nuclei and neural lobe but not in the circumvascular organs. Glucose utilization increased in the supraoptic nucleus throughout the period of dehydration. In the neural lobe, a tendency to increase started at 24 hr of water-deprivation and substantive increments were seen afterwards. In the paraventricular nucleus, subfornical organ (SFO) and organum vasculosum laminae terminalis (OVLT), the increases were observed only after 48 and 72 hr dehydration. No changes were seen in the area postrema and median eminence. These results demonstrate that water-deprivation and hypertonic saline stimulate activity of the hypothalamo-neurohypophysial system, but only water-deprivation stimulates activity of the SFO and OVLT.

OXOTOCIN mRNA LEVELS IN HYPOTHALAMIC PARAVENTRICULAR AND SUPRANUCLEAR NUCLEI (PVN/SON) DURING LACTATION IN RATS: EVIDENCE FOR MAINTENANCE BY AFFERENT STIMULI FROM THE OFFSPRING. L. H. Spinnola, R. Raghotham*, and W. R. Croyle*, Dept. of Obstetrics and Gynecology, Vanderbilt University of Tennessee-Memphis, College of Medicine, Memphis, TN 38163. The objective of the present studies was to describe in detail the changes in oxytocin (OT) mRNA in the PVN/SON that occur over time during pregnancy and lactation in the rat and to test whether afferent stimuli provided by the offspring during lactation influence these levels. The PVN and SON were microdissected from pregnant and lactating females, and RNA was extracted by the RNeasy method. OT mRNA was quantified by slot-blot hybridization, using a 25 mer oligonucleotide probe complementary to bases 912-936 of OT-neurophysin precursor mRNA (Kawata et al., Brain Res. Bull. 20: 693-697, 1988), which is T-4 polynucleotide kinase-end labelled, followed by autoradiography. Blots were stripped and then reprobed with an rat type C-5 polycrossed cDNA insert consisting of α-tubulin mRNA for normalization. The levels of OT mRNA in PVN/SON were high on pregnancy day 1, reduced during pregnancy day 1-18, and increased prior to parturition on pregnancy day 20-22. Similar elevated levels were observed throughout lactation. Compared to age-matched controls, immediate removal of offspring on day of parturition resulted in a marked decline of OT mRNA expression on day 1-18, and levels remained low for 48-72 hr. Similar results were seen after offspring removal on lactation day 8. These data suggest that OT mRNA levels in the hypothalamic magnocellular region are dependent on afferent stimuli and that the maintenance of these elevated levels during lactation is influenced by afferent stimuli provided by the offspring.
438.11

DNON BLOCKS BASAL AND OSMOTICALLY STIMULATED VASOPRESSIN RELEASE. C.D. Sladec, Dept. of Neurology and Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY, 14642.

In previous experiments, the non-specific excitatory amino acid (EAA) receptor antagonist, kynurenic acid, prevented osmotic stimulation (VP) release, but inhibited hypothalamic neurohypophyseal (HNS) exons. In order to evaluate the type of EAA receptor involved in this response, the effect of DNON (6,7-dinitroveratrine, an antagonist to both ionotropic and quisqualate EAA receptors was evaluated. VP release was measured from perfused HNS exons exposed to an increase in the NaCl concentration. NaCl concentration was sufficient to induce an NaCl- and quisqualate EAA receptors was evaluated. VP release was measured from perfused HNS exons exposed to an increase in the NaCl concentration. NaCl concentration was sufficient to induce a step increase in osmolality (290-310 mosmol/kG H2O) over 1 hour. DNON (10µM) or the DMSO vehicle (1.25 µl/ml) were added 60 min prior to NaCl induced increase in osmolality. DNON decreased VP release to the basal level (p<0.05, n=8), and VP release remained uniformly suppressed during the subsequent NaCl induced increase and decrease in osmolality. These data suggest the involvement of EAA transmitters in basal and osmotically regulated VP release from HNS exons, and indicate that non-NMDA EAA receptors are important in regulating VP release from the posterior pituitary. Supported by NIH ROI-DK-19761.

438.13

EFFECTS OF ANESTHESIA ON VASOPRESSIN (VP) AND OXYTOCIN (OT) NEURONS IN RESPONSE TO ACUTE SALT LOADING. S.W. CHIN** and W.G. NORTH, Dept. of Physiology, Dartmouth Medical School, Hanover, NH 03755.

In a previous study we anesthesed animals by a combination of Nembutal and Urethane did not significantly influence the function of VP neurons. For this study, we examined the responses of OT and VP neurons to acute salt loading in conscious rats (CON, n=8) and rats under either Nembutal (NEM, 50 mg/kg p.o. and Urethane (URE, 1.6 mg/kg p.o.) anesthesia. Fifteen minutes following the in-duction of anesthesia, NEM produced an increase in basal plasma osmolality (P<0.05, n=8), and URE did not change basal Plasma (287±2 to 289±2 mosmol/kg H2O). Neither anesthetic was noted in any significant changes in basal plasma levels of oxytocin-associated neuropeptide and vasopressin-associated neuropeptide (OT-RP, VP-RP). In response to intravenous infusion of 18% saline, all three groups of rats had similar responses in Plasma. The slopes of the relationship between plasma OT-RP and Plasma were not significantly different between the CON and the NEM groups, while that for the URE group was significantly (p<0.05) smaller than the CON group (CON=10.9±1.5; NEM=9.3±1.5; URE=6.3±0.7 femol/ml/osmol/kg H2O). The slopes of the relationship between Plasma OT-RP and Plasma in the URE group were significantly less reduced than in the other two groups. Our data suggest that anesthesia induced by either Nembutal or Urethane significantly reduces the responsiveness of VP neurons to acute salt loading, but the responsiveness of OT neurons is lowered by Urethane only.

438.15

BILABELLED NEURONS OF CARASSIUS AURATI PROJECTING TO THE UROPHYSIS: DOUBLE LABELED FOR UROGENIUS I OR II IMMUNOCYTOCHEMISTRY. J.N. Fryer & S.A. Johnston, Dept. of Anat., University of Ottawa, Canada K1H 8M5.

The caudal neurosecretory system of teleost fish and its neuroendocrine organ, the urophysis, elaborate two unique neuropeptides urensis I (UII) and urensis II (UII). Spinal cord neurons projecting to the urophysis were retrogradely labelled following application of diI (Molecular Probes) crystals to the urophysis of goldfish perfused with 4% paraformaldehyde. The crystals were hypothalamic and attached caudal spinal cord (2-3cm) were sealed in a glass container for 20 wk at 23°C in the dark. Vibratome sections (40 µm; horizontal plane) were fixed in 2% glutaraldehyde and 1% paraformaldehyde in the dark (37°C) and photographed (rhodamine or fluorescein filter, Elbergh B9). Cell groups were identified following Nissl staining of the sections. All the diI-labelled cell populations occurred within 3 mm from the urophysis. The more caudal diI-labelled cells varied in size and were in dense clusters. The more rostral cells were magnocellular (30 µm) and diffusely distributed. The magnocellular neurons were not labelled with diI. Rostral to the urophysis, many somata, fiber and terminal distributions of UII- or UII-labelled a portion of the diI-labelled cell populations occurred within 3 mm from the urophysis. In conclusion, many caudal spinal cord cells project to the urophysis; a large population of these have been confirmed to be UII or UII positive; and UII neurons are widely distributed in the caudal neurosecretory system or elsewhere within the CNS. (Supported by MRC Canada)

438.16

A BIMODAL RHYTHM OF OXYTOCIN IN THE ANTERIOR PITUITARY GLAND. B. J. Aver* and M. E. Freeman, Dept. Biol. Sci., Florida State University, Tallahassee, FL 32306

We have recently proposed that oxytocin (OT) is the neurohorome responsible for the endogenous circulatory response to prolactin (PRL) secretion (Aver and Freeman, Endocrinology, 124:878, 1989). The ESR is a bimodal rhythm with a nocturnal (N) component that peaks at 0300 h and a diurnal (D) component that peaks at 1700 h.

In this study, we have investigated the concentration of OT in the anterior (AF) and posterior (PP) pituitary as well as in the peripheral circulation in order to determine if OT is responsible for stimulating PRL secretion during the periods of the ESR. Ovariectomized rats were decapitated every 2 h over a 24 h period. Each AF or PP was extracted with 2 N acetic acid. OT was assayed by RIA. Extracts from both the AP and PP caused displacement which was parallel with ESR standards in the OT RIA. The AP contained a bimodal rhythm of OT concentration. Low levels of OT were present at 1200 h and 2200 h. A N surge began at 2400 h and peaked at 0200 h. OT concentrations at 0200 h were 6-fold greater than at 1200 h (p<0.01). Another, smaller D surge of OT in the AP began at 1700 h and peaked at 2000 h. The OT concentration of the AP at 2000 h was 5-fold greater than at 1200 h (p<0.05). Both OT peaks were also seen in the PP. To decline to baseline at 2200 h. There was no detectable diurnal rhythm of OT in either the PP or peripheral circulation. Taken together, these data suggest that OT is secreted into the AP in a bimodal rhythmic fashion with a periodicity coincident with the periodicity of the ESR for PRL secretion. Furthermore, it provides further evidence for a role of OT as the neurohorome responsible for the ESR. Supported by NIH, HD-11669.

438.17

NIGHTTIME PEAK OF OXYTOCIN IN THE POSTERIOR PITUITARY. M. J. Hanley, Dept. of Zoology, University of Ottawa, Ottawa, Ontario, Canada.

The concentration of oxytocin (OT) in the posterior pituitary (PP) was measured by RIA in 24 h cycles in 9 experiments in the Department of Zoology, University of Ottawa. The OT concentration in the PP was significantly higher at 1700 h than at 0900 h (p<0.01). This suggests that the OT in the PP is produced either by a bimodal rhythm with peaks at 1700 h and 0900 h or by a circadian rhythm with peaks at 2400 h and 0600 h. The OT peak at 1700 h is consistent with the observed peak of OT in the ESR.
438.1


The E status of the female rat affects its endocrine response to stress. To explore this effect, we measured plasma ACTH and CORT, MR, and MR mRNA in 21 day OVX and OVX rats given E for 21 days. Pre- and post-stress CORT levels were decreased in injected rats vs. controls. Dose blood sampling revealed a time-limited interaction (p < 0.04) found following footshock. Both ACTH and CORT were lower (p < 0.05) at 60 min in E treated rats. This suggested an impairment of the CORT negative feedback mechanisms. We measured glucocorticoid receptor and MR levels in various brain regions with an in vitro binding assay using 3H-CORT, R 26362, and dexamethasone. E treated rats showed elevated (p < 0.05) levels of MR in the hippocampus (HIPP) and hypothalamus. Prenocipital area (POA) and type I mRNA levels were measured with an RIA protection assay using a 180 bp cRNA transcribed from preMg. In OVX rats there were 4.65.5 fmol MR mRNA/pg RNA in HIPP and 1.6<0.1 fmol/mg RNA in POA. E treatment for 1 day lowered MR mRNA levels to 3.8 0.5 fmol/mg RNA in HIPP and to 0.6<0.1 fmol/mg RNA in POA. 21 days of E treatment lowered MR mRNA levels to 2.4 0.2 fmol/mg RNA in HIPP. These data suggest that alterations in MR may underlie the changes in ACTH and CORT secretion seen in E treated rats.

438.2


The dissociation constant (Kd) of the mineralocorticoid receptor (MR) in the hippocampus and hypothalamus is several fold greater in female than in male rats. This study addresses the possibility that the observed affinity difference is due to the presence of progesterone in females. Twenty-four adult, female Sprague-Dawley rats were neither treated nor injected at for 10 days with vehicle alone, estrogen (100pg), or estrogen plus either a low (50ng) or high (500ng) dose of progesterone. Animals were aedricenterized 20 hr before being killed under anesthesia by cardiac perfusion. Multiple blood samples were taken for determination of plasma progesterone. Binding parameters of solvable receptors have been measured in hippocampus and kidney slices of these animals by LH-20 gel electrophoresis.

Saturations plots of MR using 3H-dexamethasone in the presence of excess RU 28362 in kidney cytosol showed a significant difference in Kd between the vehicle control group and that receiving high dose progesterone (2.39 0.40 vs. 6.08 1.54, p < 0.05). For kidney, the Kd for estrogen-treated group (2.43 0.31) was similar to control, and that for the low dose progesterone group (3.71 0.45) was similar to the control, while that for the high dose progesterone group (121 24 352 18 fm/mg protein). The data indicate that the sex difference is the dissociation constant of the MR in hippocampus is not due to the action of progesterone in promoting dissociation of the MR-ligand complex. Supported by NIH 21528.

439.3


Recently we have shown that serotonin (5-HT) regulates the development of rat hippocampal type II corticosteroid receptors (CSR). We have reported, in dispersed hippocampal cell cultures prepared from fetal rat (E19-20), 5-HT increased type II CSR binding (receptor binding measured using 3HJRU 28362). This effect required a minimum of 4 days exposure to 5-HT. The effect of 5-HT was mimicked by 5-HT2 receptor agonists and blocked by 5-HT2 receptor antagonists, such as ketanserin and mianserin. The present studies used this in vitro model to further explore 5-HT induction of type II CSR binding. Cultures exposed to 10 nM 5-HT for 7 days showed a significant (p < 0.01) increase in type II CSR binding that persisted for at least 30 days following the removal of 5-HT, thus mimicking the long-term development effects seen in vivo. Cultures treated with 10 nM 5-HT showed a significant elevation of cAMP levels (400%) that persisted for 7 days following 5-HT treatment. The effect was blocked by 100 nM ketanserin, and mimicked by 5-HT2 receptor agonists. Treatment of cultured hippocampal cells with the CAMP analog, 8-bromo cAMP, for 4 days produced a dose-related increase in type II CSR binding. The effect of 8-bromo cAMP was maximal at 10 µM concentrations, and the magnitude of the effect was comparable to that seen for 5-HT (140% vs. 18%). Preliminary data indicate that activation of endogenous CAMP by forskolin treatment also increases type II CSR binding. Taken together with our previous findings, these results suggest that 5-HT is involved in the development of type II CSR binding and that the effect of 5-HT may mediated by 5-HT-induced increases in cAMP levels.

439.4


Behavioral and cardiovascular effects of centrally given CRF appeared to depend on adrenal hormones. Administration of the mineralocorticoid (RU 28318) and the glucocorticoid antagonist (RU 38486) 60 min before infusion of CRF were employed to investigate the role of the two receptor types on the CRF induced hemorrhagic and cardiovascular changes. The antagonists were administered i.c.v. at a dose of 100ng/rat. CRF infusion itself (i.c.v., 300ng/rat) caused an elevation of plasma corticosterone (CS) and norepinephrine (NE) in combination with higher blood-pressure and heart rate. Prior treatment with the anti-glucocorticoid led to a matched enhancement of the NE response, and slight increase in the CS response. In addition, the increase in plasma norepinephrine levels was seen, without an effect on blood pressure. The antianinaner- aldosterone receptor regulated processes lead to a very selective modulation of CRF-induced endocrine and physiological responses. This study was in part supported by the Foundation for Medical and Health Research HEIDION (grant nr. 90-511-044).
IMMUNOCYTOTOCHEMICAL DEMONSTRATION OF MINERALOCORTICOID TYPE I RECEPTORS IN PRIMAR Y BRAIN CELL CULTURES. Y.C. Chou, Z. S. Kowalski, W. G. Lutter and A. Saven. Department of Neuroendocrinology and Physiology, University of Florida, Gainesville, FL 32610 and Medical Research Center, Prince Henry's Hospital, Melbourne, Australia 3004

It is now well documented that mineralocorticoids, acting via specific receptors, have profound metabolic, neuroendocrine and behavioral effects on the brain. This lab has demonstrated a high binding to mineralocorticoid Type I receptors in astrocyte glial cultures similar to the binding found in brain and peripheral tissues. By using a polyclonal antiserum against a synthetic peptide (MINRECC), we have found that the binding to the enzyme the human mineralocorticoid receptor, we have observed immunoreactive Type I receptors in a variety of brain cell cultures. MINRECC antigenicity, detected by indirect alkaline phosphatase immunostaining, was found in both nuclei and cytoplasm of cells in primary cultures containing 90% neurons, astrocyte glial culture and "mixed" cultures (containing 50% neurons/50% astrocytes). Both neurons and astrocyte glia displayed immunoreactive Type I receptors. Because different types of cultures were grown using different sera, e.g. plasma-derived horse serum (PDHS) (neurons) and fetal bovine serum (FBS) (astrocytes), it was important to examine the effects of these sera on the expression of Type I receptors in cultured cells. It appears that the immunostaining observed in neurons maintained in 10% PDHS is not different from that in 5% PDHS/5% FBS. The presence of MINRECC staining in cultured neurons and astrocyte glia suggest that in addition to the ligand binding properties, Type I receptors in these cells are also similar in structure, at least in the hind region, with those seen in brain and peripheral tissues. (Supported by NIH grants NS-19441 and HL-56645.)

ROLE OF GTP BINDING PROTEINS IN ADRENAL-INDUCED CHANGES IN HIPPOCAMPAL CALMODULIN ADENYLYL CYCLASE ACTIVITY. H. N. Gannon, T. Akompong and B. S. McEwen. Laboratory of Neuroendocrinology, Rockefeller University, New York, NY 10021.

We have observed that adrenalin (AD), the major adrenoceptor agonist, stimulates calmodulin (CaM) and forskolin (F) stimulated adenylyl cyclase (AC) activity in cultures of hippocampal (HC) membranes. This effect is prevented by high, but not low, corticosterone replacement in ADX rats. AD changes have been reported to alter levels of specific GTP binding (G) proteins (Gsa and G3a) in rat cortex, an effect prevented by CORT replacement. (Saio, N., et al., PNAS, 1989). These results indicate a role for G proteins in ADX effects on HC AC activity. In both sham and ADX HC membranes incubation with the G-protein antagonist, GDP-PS, did not abolish ADX attenuation of CaM, but decreased CaM more than basal or F-AC activity. Substitution of Mg2+ for Mg2+ in the AC assay, which selectively uncouples G protein control of AC, abolished CaM stimulation, but ADX still attenuated F AC. ADX had no significant effect on HC membrane Gsa and G3a content, measured by Western blot method. These results suggest that ADX effects on HC AC may not occur through modulation of G-protein(s) levels, despite the fact that HC AC appears selectively dependent on G-protein(s). Supported by NHLBI-41256.


Both glucocorticoid receptors (GR) Type I and II exist in various extrahypothalamic regions, in particular the hippocampus, and have been implicated in the basal and stress-associated negative feedback control of the hypothalamic-pituitary-adrenal (HPA) axis. We have investigated the effects of altered thyroid status on the intracellular glucocorticoid type I receptor concentrations in the hippocampus of adult male SD rats with short- (7 days) or long-standing (60 days) hypothyroidism (thyroidectomy + placebo), euthyroidism (sham thyroidectomy + placebo) or hyperthyroidism (thyroidectomy + thyroxine, 50 mug/day). We measured hippocampal GC Kds and concentrations and plasma concentrations of IR-AC and IR-corticosterone (B). There were no significant changes observed in the DEX binding as a function of thyroid status or duration of treatment. Although hypothryroid rats exhibited no changes in intracellular GC concentrations, however, hyperthyroid rats had significantly lower concentrations of glucocorticoid receptors both at 7 and 60 days of thyroxine treatment (p<0.001). Plasma ACTH and B levels increased in hyperthyroid animals, suggesting increased HPA axis activity in these animals. These data indicate that thyroid hormones modulate hippocampal GC type II concentrations; and increased HPA axis activity in hypothyroid states can be explained by a lower number of GR and decreased glucocorticoid feedback inhibition.


An ultradian rhythm in corticosterone (B) secretion which is synchronized between animals has been demonstrated in the rat using intra-adrenal microdialysis (FASER J. 4:287-90,1990). To determine the effect of the splanchnicotomy of the adrenal on rhythmic secretions of B, intra-adrenal microdialysis was done after splanchicotomy (SPX). Under pentobarbital anesthesia, adult male rats were prepared with a jugular vein catheter and an adrenal probe constructed from cellulose fibers (0.4D MW cutoff). In SPX rats (n=6), the thecal-intact plasma peak cut proximal to the suprarenal ganglion. In sham rats (n=7), no surge was cut. Experiments conducted 1 day post-surgery consisted of continuous collection of dialysate at 10 min intervals (flow rate=10 pl/min) from 1000 to 1800 hr, with blood sampling at 1030 and 1730 hr. Dialysate B and plasma B were measured by RIA. Plasma B was similar in both groups. Episodic secretion of B persisted after SPX. Time domain analysis using PC- Pulsar revealed no difference in pulse frequency, width, inter-peak intervals of the normalized pulse amplitude. To determine if underlying periodic components in B secretion might be altered by SPX, frequency spectra were calculated for individual rats by Fourier analysis. Averaging individual spectra within groups revealed peaks at approximately 60 and 30 min in sham rats. In SPX rats, the 60 min peak remained, whereas the 30 min peak was attenuated; additional energy was observed between 90 and 200 min in SPX rats. Averaging across time-series before spectral analysis did not change the spectral profile, indicating that episodes are synchronized between animals in SPX and in sham rats. These data suggest that adrenal splanchic innervation modulates ultradian rhythms of adrenal B secretion, but does not directly generate or synchronize these rhythmic episodes. Supported by NIH grant DK38951.

EFFECTS OF AGING ON THE HIPPOCAMPAL glucocorticoid and MINERALOCORTICOID RECEPTORS. M.I. Morano and H. Askl. Mental Health Research Institute, The University of Michigan, MI 48109-0720.

The basal levels of plasma corticosterone do not differ between young and old animals. However, the recovery to basal corticosterone levels following the termination of restraint stress is delayed in old rats. We have measured the cytosolic MR and GR binding capacity as well as the expression of the mRNA's of both the receptors in the hippocampus of young (5 mo.) and old (28 mo.) F-344 rats. By demonstrating the role of MR and GR in the hippocampus of old rats (57 and 52 % of those in young ones, respectively), we have demonstrated that the expression of mineralocorticoid receptors in old rats is lower than in young ones. The mRNA contents of MR and GR are lower in old sham-operated rats than in the young ones (p < 0.05). Interestingly, following adrenalectomy, only the old animals showed up-regulation of the levels of these mRNAs reaching levels comparable to the young; however, the binding capacities of these receptors in old rats did not achieve the levels in young rats. These data point to a possible change in mRNA stability and translatability in aged animals or to an alteration of steroid receptor cell cycle with age. Supported by MH 42251, DA 02265 and Markey Foundation.

94.10

MR-801 ANTAGONIZES MAMMOTHYMPHINE-INDECREASSE IN NEUROHICAL corticosteroiD RECEPTORS. M.T. Lowy, Dept. of Psychiatry, Case Western Reserve University Sch. of Med., Cleveland, OH 44106.

Previous work in this laboratory demonstrated that depletion of biogenic amines by reserpine decreases hippocampal type II corticosteroid receptors (CR) (Lowy, 1990). The present study was designed to determine if methamphetamine (MA), a stimulant drug which is toxic to serotonin containing neuronal systems, decreases neuronal levels of CR. Administration of MA (15 mg/kg) to 1 day adrenalectomized rats decreased hippocampal type I (33%) and II (-15%) CR type II (-21%) measured 3 hrs later. Cortical and hypothalamic type II CR were not affected. Since previous studies have demonstrated that CR mRNA antagonized the MA-induced decrease in hippocampal type I and II CR as well as stratal type II CR. To directly examine if excitatory amino acids can regulate hippocampal CR, MR-801 antagonized the MA-induced decrease in hippocampal type I and II CR as well as stratal type II CR. These experiments indicate that MA decreases neuronal CR via a NMDA sensitive mechanism and that excitatory amino acids may play a role in hippocampal CR regulation.
440.1 PERIPHERAL NERVE INJURY CAUSES CUTANEOUS NERVOUS RECEPTORS TO BE EXCITED BY ACTIVATION OF CATECHOLAMINE RECEPTORS J.-R. S. and E. R. F. Dept. of Physiol., Univ. of North Carolina, Chapel Hill, NC 27599.

We have shown that after nerve injury but not in normal animals, sympathetic trunk stimulation (55) or intraarterial catecholamine administration (NE) causes a fraction of C-fiber polymodal nociceptors (CPN) to discharge or to show enhanced heat-evoked sensitization. To test whether these effects on nociceptor activity are mediated by peripheral catecholamine receptors, the great auricular nerve (GAN) of deeply-anesthetized domestic rabbits was partially damaged under sterile conditions and the animals allowed to recover for 10 to 24 days. Post-operatively, some of the animals exhibited signs of decapsulation of terminal or, under deep anesthesia, responses of CPN units to SS, NE and stereotyped heat stimuli were recorded from GAN neurons central to the site of damage. The relatively specific α₁ agonists, yohimbine or rauwolscine (0.1-3 mg/kg) reduced or blocked the GAN excitation by SS and NE for 60-180 min, and reduced CPN sensitization by heat to 60% of control values. These results suggest that sympathetic and NE excitation of nociceptors after nerve injury probably is mediated by α₁ sensitive receptors, phenomena possibly related to the pathogenesis of some causalgia-like pain syndromes. (Supported by grants NS 10321 and 16489 from NINDS.)

440.3 EVIDENCE THAT FAST AXONAL TRANSPORT IS INVOLVED IN THE DEVELOPMENT OF MECHANOSENSITIVITY IN AN ACUTELY CUT NERVE. G.M. Kosharke*, R.A. Meyer, and J.N. Campbell. The Johns Hopkins Univ., Baltimore, MD 21205.

Severed nerves develop mechanosensitivity at their ligated ends within hours of injury. The rate of development of mechanosensitivity was temperature sensitive. Because axonal transport is similarly temperature sensitive, we postulated that axonal transport plays an important role in the development of ectopic excitability. To test this further and to determine if fast or slow axonal transport is involved, the sural nerve in anesthetized monkeys (macaca fascicularis) was tightly ligated at a proximal site to cut off the source of transported substances. At a site 85 mm distal, the nerve was tightly ligated and cut either 3 or 12 hours later. At 3 hr, but not at 12 hr, substances conveyed by fast transport would still be in the nerve between the ligation sites. Ten hours after the distal injury, action potential activity in A-fibers was recorded in response to a mechanical stimulus at the distal site. Only 3% of the A-fibers responded to mechanical stimulation for the 12 hr experiment, whereas 20% of A-fibers responded for the 3 hr experiment. These results are consistent with the hypothesis that the components required for mechanical-to-electrical transduction at sensory receptors are conveyed via fast axonal transport at the nerve injury site to impart ectopic excitability. (Supported by NIH NS-14477 and DOD N00039-90-C-3501)


Thoracic dorsal rhizotomy (TD) causes pronounced ventilatory failure in goats during mild exercise, followed by a marked decrease in ventilation during subsequent exercise trials (Mitchell et al. Aerospace Medicine, 1988). As an initial approach to study spinal changes associated with functional deficits or recovery, we investigated the distribution of calcitonin gene-related peptide (CGRP; an axon reflex modifier for primary sensory afferents) and substance P (SP). Bilateral dorsal rhizotomies were performed from T2-T12 (n=6). Six to twelve months after surgery, the goats were sacrificed, and their spinal cords removed and immersed in a tissue freezing medium of 50 μm sections were processed for immunoreactivity using conventional PAP methodology. For comparison, sections from sham-operated and sham-operated goats were processed similarly. At thoracic levels, CGRP fibers were concentrated in laminae I and II. Fiber bundles were also seen in the medial portion of lamina V with scattered CGRP fibers in the ventral horn. TDR nearly eliminated CGRP fibers in the dorsal horn; only a thin band of fibers that skirted the underside of the dorsal horn remained. At cervical levels, however, TDR goats showed increased CGRP labeling in laminae III and IV. There were no obvious differences in CGRP immunoreactivity in the ventral horn. SP immunoreactivity paralleled the CGRP pattern in both normal and TDR goat tissue. These results indicate that even 6 months following TDR, regrowth of CGRP containing afferents does not occur, and that functional recovery of ventilatory control does not result from such regrowth. However, plasticity in the spinal cord may have occurred and contributed to long-term compensatory mechanisms as suggested by increased CGRP and SP labeling in cervical spinal cord - spinal cord regions critically involved with the control of breathing. (NIH HL36780, HL01694, NS26850 and NIEHS Training Grant T32 ES-07105).


Using a horseradish peroxidase (HRP) labeling technique, we examined whether that ventral root axons invade the spinal cord after a neonatal peripheral nerve lesion. A total of 11 anesthetized adult Sprague-Dawley rats were used (7 neonatal sciatic neurotomerized and 4 normal control rats). After the L4-L6 dorsal roots of both sides were cut, HRP (3%) was injected into the spinal cord. HRP labeled cells were counted in the L5 dorsal root ganglion (DRG).

In rats subjected to neonatal sciatic neurectomy, there was an average of 45 labeled cells in each side of the L5 DRG on the experimental side, whereas the DRG on the contralateral side contained 11 labeled neurons. On the other hand, the L5 DRG of normal control rats contained an average of 1 labeled neuron. The average size of labeled cells (29±11), was significantly smaller than the size of random sampled DRG cells (34±11).

These results suggest that lesioning the sciatic nerve during the neonatal stage triggers sprouting of fibers of small DRG cells, and that these fibers invade the cord via the ventral root. (Supported by NIH grants NS21266 & NS11255 and a grant from Bristol-Miers Co.)

440.6 BEHAVIORAL EVIDENCE FOR THE DEVELOPMENT OF TRIGEMINAL NEUROPATHIC PAIN FOLLOWING LIGATION OF THE INFRAOBIAL NERVE IN THE RAT. B.P. Vos and R. Macler, Phys. Physiology Lab. Dept. of Neurology, Massachusetts General Hospital, Charlestown, MA 02129.

Loosely ligating the rat's trigeminal nerve produces behavioral changes consistent with neuropathic pain in the affected hind limb (Bennett & Xie, Pain, 1988). To develop an experimental model of trigeminal neuropathic pain, we ligated the infracranial nerve (ION) in a similar fashion in the rat, and studied behavioral changes related to facial somatosensory function.

Experimental rats receiving ION ligation demonstrated a contralateral sham operation. In control rats a unilateral sham operation was done. We quantified face grooming behavior, trigeminal tract scarring activity, and responses to mechanical stimulation (tactile, vibratory, pinch). Behavioral changes were observed. These included bilateral changes consistent with neuropathic pain in the affected hind limb (Bennett & Xie, Pain, 1988). To develop an experimental model of trigeminal neuropathic pain, we ligated the infraorbital nerve (ION) in a similar fashion in the rat, and studied behavioral changes related to facial somatosensory function.

Experimental rats receiving ION ligation demonstrated a contralateral sham operation. In control rats a unilateral sham operation was done. We quantified face grooming behavior, trigeminal tract scarring activity, and responses to mechanical stimulation (tactile, vibratory, pinch). Behavioral changes were observed. These included bilateral changes consistent with neuropathic pain in the affected hind limb (Bennett & Xie, Pain, 1988). To develop an experimental model of trigeminal neuropathic pain, we ligated the infraorbital nerve (ION) in a similar fashion in the rat, and studied behavioral changes related to facial somatosensory function.
440.7 CHANGES IN PCP BINDING SITES IN RAT SPINAL CORD IN A CHRONIC CONDUCTION INJURY J. M. Ammons, S. I. Shen, K. C. Krainev, G. L. Bennett and V. S. Seybold. Biology Dept., Macalester College, St. Paul, MN 55105; Departments of Neuroscience and Cell Biology & Neuroanatomy, University of Minnesota, Minneapolis, MN 55455; NAB, NIDR, NIH, Bethesda, MD 20892.

A chronic conduction injury of the sciatic nerve in rats has been proposed as a model of peripheral neuropathy (Bennett and Xie, 1988). The injury, which is induced by tying loose ligatures around the sciatic nerve, results in hyperalgesia to chemical and thermal stimuli by 4 weeks. Previous studies have shown that there is modulation of opioid and SP binding sites in this model (Ammons et al., NS Abstr. 15:103, 1988). In the present study, we examined changes in PCP receptor binding sites in lamina I/II in rat spinal cord.

Spinal segment L4 was obtained from control rats (n=5) and from rats 2,5, and 10 days after nerve ligation (n=4 each group). Autoradiographic studies were performed on spinal cord sections using 3H-PCP, nonspecific binding was determined by incubating sections with 30 uM PCP (Ammons and Seybold, 1989). Data were analyzed using computerized grain counting. Specific binding was determined by subtracting nonspecific binding from total binding in each area analyzed. Statistical analyses (one-way ANOVA with Dunnet's post-hoc test) revealed a significant change in 3H-PCP grain density in lamina I/II on the side ipsilateral to the lesion, but not on the side contralateral to the lesion. A significant increase in 3H-PCP grain density was observed on day 2 while a significant decrease was observed 10 days after nerve injury. The PCP binding 2 days after injection of adenosin on calcium channel blocker D(CGPRP (0.1 nM) and D(2S)P (0.5 nM) were used to label binding sites on tissue sections of spinal segment L4 from each animal.

Incorporation of adjacent tissue sections in radiolabeled peptide with 0.1 uM peptide was used to determine nonspecific binding. The density of autoradiographic grains within the emulsion overlaying laminae I/II of dorsal horn within each tissue section was quantified by computerized image processing. The amount of [125I]J-CGRP bound in laminae I/II decreased ipsilaterally to the injured paw at 4 days after injection of adjuvant. In contrast, the amount of [125I]J-BP bound did not change within this region at the time points examined. These data indicate that the release of CGRP within lamina I/II of the dorsal spinal cord is increased in conjunction with acute inflammation. Studies funded by NS17702.

440.11 TOWARD SELECTIVE LESIONING OF MOUSE NOCICEPTIVE DORSAL ROOT GANGLION NEURONS BY CYTOSORBENT-ATTACHED LASER PHOTOLYSIS. J.D. Maclain and L.C. Dang*. Department of Neurology, Program in Neuroscience, Harvard Medical School, The Children's Hospital, Boston, MA 02115.

Recent studies have demonstrated selective, noninvasive lesions to subpopulations of CNS neurons targeted for laser photolysis of nanoparticles carrying photoactive chromophores. Similar mechanisms are investigated here as a potential approach for selective lesioning of nociceptive neurons located in mouse dorsal root ganglia. Selective neuronal targeting, photolytic single oxygen production within labeled cells, and laser-tissue interaction were studied.

Mice were injected subcutaneously in one hindlimb with 250 uL of nanoparticles with incorporated chlorin e6. Following survival times of 1 to 12 weeks, mice were perfused and evaluated histologically for the level and distribution of labeled within neurons of different diameters. Quantitative analysis of the intracellular chromophore concentration was performed using an imaging model of neuronal labeling and a microassay for cytotoxic singlet oxygen production. Transmission of 670 nm wavelength laser energy through parapinal bone and soft tissue was measured directly and compared with the solution of an appropriate analytical model using the diffusion approximation equation.

Dorsal root ganglia ipsilateral to the injury sites displayed intraneuronal labeling predominantly within neurons 10-12 uM diameter. These results suggest that these new methods could effectively lesion 10-12 uM diameter neurons. Supported by NS38279, HD00659, and the Alzheimer's Association.

440.8 CHANGES IN [125I]J-CGRP BINDING SITES IN THE DORSAL HORN OF RAT SPINAL CORD FOLLOWING DORSAL RHIZOTOMY. M.G. Garry and V.S. Seybold. Dept. of Cell Biology and Neuroanatomy, Grad. Program in Neuroscience, University of Minnesota, Minneapolis, MN 55455.

Whereas receptor binding sites for many peptides have been described in the brain and spinal cord, there have been limited reports addressing the regulation of binding sites for neuropeptides (other than the opioids) within the central nervous system. Calcium-activated peptide receptor (CGRP) is a primaryafferent neuropeptide at the spinal level, although the role of CGRP in spinal processing is equivocal. In order to determine whether dorsal horn cells regulate their binding sites for CGRP, we have used a computerized laser photolysis method and an axotomy model to investigate changes in CGRP binding sites in the dorsal horn. Binding was analyzed using a computerized imaging method and a laser photolysis method. After axotomy of the dorsal horn in rats, unmyelinated neurons in any of the laminae examined. On the intact side of the experimental animals, low densities of binding sites were observed in the superficial laminae. Selective, peripheral deafferentation, however, significantly increases in the amount of CGRP binding were observed at 4 and 8 days following rhizotomy. These changes were observed at the medio-lateral aspect of laminae I/II and in lamina V (3-way ANOVA, p<0.05). No changes were observed in lamina X.

These results indicate that CGRP binding sites are regulated by the extracellular concentration of CGRP. In addition, these data suggest that CGRP binding sites may normally be "down-regulated" due to basal levels of CGRP release.
440.13
STREPTOZOTOCIN-DIABETIC ANIMALS DISPLAY A HYPERALGESIA OF NON-PARGOSTALGIND ORIGIN
J.C. Eldred and L. B. Hurd. Department of Biology, Boston University, Boston, MA 02215. We used antiserum directed against serotonin to label select populations of bipolar and amacrine cells in the turtle retina (Pseudemys scripta elegans). The processes of labeled bipolar cells in the outer plexiform layer were commonly seen to make wide gap contacts with photoreceptors. Processes of serotonergic bipolar cells were rarely seen near photoreceptor ribbons. Labeled bipolar cells formed dyads onto either amacrine/amacrine cell or amacrine/ganglion cell pairs in stratum 1 of the inner plexiform layer. Outputs of bipolar cells in strata 4/5 were similar in type, but fewer in number than in stratum 1. Inputs to both labeled bipolar and amacrine cells in strata 1 and 4/5 were from unlabeled amacrine cells. Serotonergic amacrine cells made synapses onto unlabeled amacrine and ganglion cells in strata 1 and 4/5. Rare synaptic contacts from labeled amacrine cells onto unlabeled amacrine and ganglion cells were seen in the region of stratum 3. These results support the role of serotonergic inputs to OFF, photoreceptor, amacrine, and ganglion cells in the turtle retina, and provide data on the synaptic interactions of the serotonergic amacrine cells in the IFL. This research supported by EY04785 to WDE.

440.14
THE NUMBER OF SUBSTANCE P CONTAINING DORSAL ROOT GANGLION CELLS IS REDUCED AFTER LASER IRRADIATION OF THE RAT TIBIAL NERVE. L. Weissmann and W. Z. Rymer. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611. Recent studies from our laboratory indicate that Q-switched Nd:YAG laser irradiation might have selective effects on properties of small sensory nerve fibers. We have previously demonstrated that laser irradiation selectively impairs neuronal impulse propagation in small saphenous conveying fibres (Wessman et al., Soc. Neuroscience Abstr. 14:698, 1988). In addition we have shown that the number of small DRG cells labeled with HRP is selectively reduced after laser irradiation of the rat tibial nerve, while the number of large sensory neurons is not affected (Wessman et al., Soc. Neuroscience Abstr. 15:102, 1989). Substance P (SP) is a neurotransmitter, that has been shown to occur preferentially in the cell bodies of small sensory neurons. In an attempt to further analyze the preferential effect of laser application on small sensory nerve fibers, we have studied the effects of laser irradiation (Q-switched Nd:YAG laser, pulse energy -70 mJ/pulse, irradiation time - 5 min) of the rat tibial nerve on the number of SP-labeled sensory neurons in DRG L4 and L5. In control animals (n=4) the numbers of SP containing neurons were not significantly different between sides (left: 4404,404, right: 4450,4,397)*. In contrast, after previous laser irradiation of the peripheral nerve the number of SP containing DRG cells was significantly reduced on the laser-treated side (left: 2710,327, right: 4450,4,342, P<0.001)*. The functional significance of this finding remains to be examined electrophysiologically. Since a large number of SP containing neurons appear to have nociceptive functions, the selective effects of laser irradiation on this neuron class might lead to potentially useful applications for the treatment of chronic pain. (Supported by the Medical FEL Program ONR/SDIO N00014-86-K-0188) *mean±SEM.

440.15
INCREASES IN GFAP IMMUNOSTAINING IN THE LUMBAR SPINAL CORD FOLLOWING SCIAITIC NERVE INJURY. C.Garrison, P.Houghton, K.Jajander and S.M.Carlson. University of Minnesota, TX Medical Branch & Marine Biomedical Institute, Galveston, TX 77550. Recent studies indicate that glial cells within the spinal cord respond to transection of a peripheral nerve. Although the signal for this response remains unknown, evidence supports a neurochemical mediation of this phenomenon. Neurochemical changes occur in the spinal cord gray matter in the animal model of experimental peripheral neuropathy (EPN). It is unknown if glial responses also result from this nerve insult. Therefore, in the following study, EPN was induced in one hindlimb of 3 rats; the contralateral limb received a sham operation. Post-surgical day 10, animals were perfused with 4% paraformaldehyde and L4-5 segments removed. The tissue was frozen sectioned (25um) immunostained with glial fibrillary acidic protein (GFAP). Counting reveals a significant difference in the number of GFAP immunostained cells in experimental versus control horns; however, measurements of grey matter density demonstrated a significant increase in staining density on the experimental side. In addition to further characterizing spinal cord changes in the EPN model, these findings confirm that astrocytes respond to peripheral nerve injury, possibly playing a role in nervous system pathology. Supported by NIH grant NS21647.

441.1
SYNAPTIC ANALYSIS OF SEROTONERGIC NEURONS IN THE TURTLE RETINA. W. D. Eldred and L. B. Hurd. Department of Biology, Boston University, Boston, MA 02215. The processes of labeled bipolar cells in the outer plexiform layer were commonly seen to make wide gap contacts with photoreceptors. Processes of serotonergic bipolar cells were rarely seen near photoreceptor ribbons. Labeled bipolar cells formed dyads onto either amacrine/amacrine cell or amacrine/ganglion cell pairs in stratum 1 of the inner plexiform layer. Outputs of bipolar cells in strata 4/5 were similar in type, but fewer in number than in stratum 1. Inputs to both labeled bipolar and amacrine cells in strata 1 and 4/5 were from unlabeled amacrine cells. Serotonergic amacrine cells made synapses onto unlabeled amacrine and ganglion cells in strata 1 and 4/5. Rare synaptic contacts from labeled amacrine cells onto unlabeled amacrine and ganglion cells were seen in the region of stratum 3. These results support the role of serotonergic inputs to OFF, photoreceptor, amacrine, and ganglion cells in the turtle retina, and provide data on the synaptic interactions of the serotonergic amacrine cells in the IPL. This research supported by EY04785 to WDE.

441.2
LOCALIZATION OF SEROTONIN UPTAKE MECHANISMS IN RABBIT RETINA. W.J. Brunken and C.P. Zimelidine. Department of Biology, Boston College, Boston, MA 02167. In mammalian retina, uptake mechanisms have been used as markers of indolamineogenic function. Previous kinetic analysis has demonstrated that two mechanisms with different affinities are present. Anatomically three classes of amacrine cells have been shown to take up 5HT. In this study, we have begun to localize these transport mechanisms to anatomically identified cell classes. Rabbit retinas were exposed to exogenous 5HT in the presence or absence of zimelidine, an uptake blocker. In control experiments, immunohistochemical demonstration of uptake at low doses of 5HT revealed a single plaque of fibers in the IPL and occasional amacrine cells (2.06 cells/mm2); at higher doses of 5HT, the density of labeled cells increased (5.97 cells/mm2) and the IPL staining was diffused. Zimelidine was able to reduce by 25 to 50% the density of labeled cells depending on the concentration of exogenous 5HT. Together these data suggest that both the low and high affinity transport mechanisms may be localized in different cell classes. Kinetic analysis of 5-HT uptake supports this hypothesis. We have confirmed the existence of two classes of high affinity (ca 10M) and one with low affinity (ca 10 M). Zimelidine reduced uptake by the low affinity transporter but did not affect the high affinity transport mechanism. Zimelidine also reduced the maximal uptake of 5HT by the retina at 45 and 60 minutes by 42 and 55% respectively. Finally, we used 5HT-paroxetine to label the high affinity transporter. Scatchard plots revealed a single binding site with a KD of 285 binding protein and a Kd of 0.35 nM. Zimelidine did not displace paroxetine binding suggesting that it is effective only at the low affinity transporter. These data suggest that only those cells labelled with low doses of exogenous 5HT or in the presence of zimelidine possess the high affinity 5HT transporter.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
441.3 DOUBLE-LABEL ANALYSIS OF THE COEXISTENCE OF SOMATOSTATIN AND NEUROTENSIN IN AMACINE CELLS OF THE CHICKEN RETINA. C.B. Watt, H.B. Li* and P.A. Bizeynek

Alice R. McPherson Laboratory of Retina Research, The Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381.

A comparative immunocytochemical study revealed a striking similarity in the morphology of populations of somatostatin and neurotensinergic amacrine cells in the chicken retina. A double-label study was performed to determine if the neurotensin- and somatostatinergic peptides coexist in amacrine cell bodies. A routine double-label paradigm was performed on cryosections collected through each quadrant (dorsolateral, dorsomedial, ventrolateral, ventromedial). Primary antibodies to the respective peptides were conjugated to fluorochrome, and the secondary antibody was conjugated to biotin or rhodamine. Control experiments testing the specificity of primary and secondary antibodies indicated no cross-reactive tendencies. Importantly, an examination of more than eight thousand labeled cells in each of the four retinal quadrants revealed that all labeled amacrine cell bodies express both somatostatin- and neurotensin-like immunoreactivity. Therefore, these results indicate the presence of a single population of amacrine cells whose members contain both of these putative neuroactive peptides.

This work was supported by NIH grant EY05622 and by the Retina Research Foundation (Houston).

441.4 POSTNATAL DEVELOPMENT OF TYROSINE HYDROXYLASE-IMMUNOREACTIVE (TH-IR) NEURONS IN THE RABBIT RETINA. Giovanni Casini, Nicholas C. Brecha, Ellen S. Takahashi and Clyde W. Oystrier, Dept. of Anatomy & Cell Biology and Medicine, UCLA, Los Angeles, CA, and School of Optometry, UAB, Birmingham, AL.

Dopaminergic amacrine cells play important modulatory roles in retinal function. The present study describes the postnatal development of TH-IR neurons in the rabbit retina. Putative dopaminergic neurons were identified either in retinal sections or in whole mount preparations by standard immunohistochemical protocols using a monoclonal antibody to TH. The TH-IR cells were observed in the inner nuclear layer between the 5th and the 7th postnatal days. These neurons displayed weak immunoreactivity, and from day 8 immunoreactive processes could be observed. In the following days, the intensity of immunoreactivity increased, and by day 12 most of the TH-IR amacrine cells showed one or two major primary processes which had secondary branches stratifying in lamina 1 of the inner plexiform layer (IPL). At day 21, TH-IR cell bodies approached the morphology and the staining intensity typical of the adult, and an almost continuous band of immunoreactivity was present in lamina 1 of IPL. Staining intensity in the IPL increased with further development, and by day 24 all processes in laminae 3 and 5 were also encountered. These observations support earlier biochemical data and show that TH-IR neurons develop postnatally, reaching a postnatal expression sufficient to modulate retinal function.

Supported by EY04067 and VA Medical Research Funds.

441.5 GABA A AND GABA B SUBUNIT IMMUNOREACTIVITIES IN THE RABBIT RETINA. Nicholas Brecha and Christine Casini

Dept. of Anatomy & Cell Biology and Medicine, CURE and Jules Stein Eye Institute, UCLA School of Medicine and VAMC-VA Medical, Los Angeles, CA.

The existence of subtypes of GABA A receptor (α, β, γ and δ) and their differential expression is consistent with the presence of multiple GABA A receptors. This study evaluated the cellular localization and distribution of two of these subunits, α and δ, in the rabbit retina using immunohistochemical techniques. Retinas were fixed in 4% paraformaldehyde and sectioned perpendicular to the retinal surface with a vibratome. Monoclonal antibody bd-24, directed to the α subunit, labels processes distributed to laminae 1, 3 and 5 of the inner plexiform layer (IPL) and to the outer plexiform layer. Some amacrine and horizontal cell bodies are heavily labeled. Faint labeling is occasionally observed in some cells located in the ganglion cell layer. Monoclonal antibodies bd-17 and 62-3GI, directed to the δ subunit, also label processes distributed to laminae 1, 3 and 5 of the IPL and a few amacrine cell bodies. No staining is observed in adjacent sections incubated in normal serum. The observation of this staining, along with earlier in situ hybridization studies, emphasizes the multiplicity and heterogeneity of GABA A receptor expression in the retina.

bd-17 and bd-24 were generously supplied by Dr. J. G. Richards; 62-3GI was generously supplied by Dr. A. L. de Blas. Supported by EY04067 and VA Medical Research Funds.

441.6 β-NEERVE GROWTH FACTOR RECEPTOR IMMUNOREACTIVITY IN THE DEVELOPING RABBIT RETINA. Dennis W. Rickman, Nicholas C. Brecha and David Davidson*, Dept. of Anatomy & Cell Biology, USC, Medicine and Anatomy & Cell Biology, UCLA, Los Angeles, CA; and Medicine, University of Bristol, U.K.

The effects of nerve growth factor (NGF) in the survival of neurite outgrowth and target innervation of developing neural crest-derived neurons are well-documented. Recent studies with radiolabeled NGF or antibodies to the NGF receptor (NGF-R) suggest that NGF may have similar effects on neuroectoderm-derived neurons in the developing CNS, including the retina. Here, we used a monoclonal antibody (ME20.4) to the NGF low-affinity β-NGF-R to examine the localization and temporal pattern of β-NGF-R expression in embryonic and postnatal rabbit retina. At embryonic day 15 (E-15) and E-16, ME20.4 labeled radially-oriented cells which extended from the ventricular edge to the inner limiting membrane. At E-29 staining was observed in Müller cells, some amacrine cell somata, numerous ganglion cell somata and axons in the ganglion cell axon layer (GAL). This staining pattern was observed throughout the early postnatal period (P-2 to P-10) with particularly heavy labeling of ganglion cell axons in the GAL. At P-21 no staining was detected in any retinal layers. These observations suggest that β-NGF-R is expressed transiently throughout the period of retinal differentiation and may play a role in the development of retinal innervation and target recognition by ganglion cell axons.

Supported by EY 04067 and VA Medical Research Funds.

441.7 Distribution patterns of parvalbumin immunoreactivity in the vertebrate retina

Pietro Paolo Scienza, Ken J. Howard, Elena Bala-Berri, Harvey J. Karten* and Floyd E. Bloom

Department of Neuropharmacology, Research Institute of Scripps Clinic and Research Foundation, La Jolla, CA 92037.

Parvalbumin is a low molecular weight calcium binding protein which has been proposed to affect the firing speed in muscle fibers and is also specific to discrete neuronal subpopulations. We have investigated the distribution of parvalbumin-like immunoreactivity (P-LIR) in the vertebrate retina, and have found that both amacrine and ganglion cells are labeled in the goldfish retina only amacrine cells were labeled. In the chicken and pigeon retina P-LIR restricted to two morphologically distinct subpopulations of amacrine cells. In the rat retina, a subset of amacrine cells and a subset of cells in the ganglion cell layer were labeled. On the basis of fluorescein retrograde tracing, the majority of the lates appeared to be ganglion cells. A similar pattern was found in the ground squirrel. In the rabbit, horizontal cells showed P-LIR as described by others (Röhrenbeck et al., Neuroscience Letters, 77 (1987) 255-260), and a subpopulation of amacrine cells as well as some sparse cells in the ganglion cell layer were also immunoreactive. In the cat retina, the majority of ganglion and horizontal cells as well as a subset of amacrine cells were labeled (as reported by Röhrenbeck and others, ibid.). In mammals, in partial confirmation of results described by others (Endo T. et al., Cell Tissue Res., 243 (1986) 21-37), horizontal and amacrine cells displayed P-LIR as did sparse cells in the ganglion cell layer.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
441.9 GAP JUNCTION DISTRIBUTION IN CAT AND MOOSE RETINA VISUALIZED WITH MONOCLONAL ANTIBODY TO CONNEXIN32. S. Vardel, E. Hertsgaard, and P. Sterling. University of Peres, Phila, PA 19104 and Albert Einstein College of Medicine, Bronx, NY 10461.

GAP junctions are known in mammalian retina from electron microscopy. The structure and function of extracellular sections however, with neither ultrastructural technique is the overall pattern of gap junctions in the tissue revealed. Immunocytochemical localization of gap junctions at the level of the light microscope by immunofluorescence using a monoclonal antibody specific for connexin32, one of several gap junction proteins thus far identified. In the outer retina, staining in the synaptic layer formed a dark band; the profiles of receptor terminals were revealed as rings by the even distribution of staining of their membranes. In the inner nuclear layer, staining was dense for connexin32 punctate deposits on horizontal cell bodies and different somas in the bipolar and amacrine cell layers. The inner photoreceptor layer was evenly distributed as fine granules. The staining pattern in monkey and cat retinae were similar. This distribution of connexin32 immunoreactivity is consistent with previous ultrastructural observations of gap junctions, but suggests the possibility of electrical coupling at sites where it has not previously been suspected (e.g. onto retinal bipolar and amacrine somas). EY 00829 (PS); OR 00667 (SU).

441.11 IS ACETYLCHOLINE INVOLVED IN LATERAL INHIBITION IN THE LIMULUS LATERAL EYE? HISTOCHEMICAL DEMONSTRATION OF ACETYLCHELSTERASE IN THE LATERAL PLEXUS. Daniel L. Sambursky, and Steven C. Chamberlain, SUNY Health Science Center at Syracuse and Institute for Sensor Research, Syracuse University, Syracuse, NY 13210.

Standard histochimical procedures for visualizing acetylcholinesterase (AChE) produced positive staining on frozen sections of the lateral eye. Deletion of acetylcholine from the lateral eye evoked release of the cholinesterase activity. On the other hand, staining was not affected by the inhibition of butyrylcholinesterase (BuChE) with Izo-OMPA. These results suggest specific demonstration of AChE rather than false localization of BuChE.

441.12 Cloning and expression of a mouse retinal potassium channel. D. D. Klumpp, D. F. Bahr and G. R. Bower* and L. H. Elion. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208 and t School of Medicine, University of California at Los Angeles, Los Angeles, CA 90024.

We have cloned mouse retinal cDNA libraries in an effort to characterize potassium channels of the mouse retina. Libraries were screened with oligonucleotide probes homologous to known potassium channels. We isolated cDNA clones spanning the complete coding regions of BRK1, a putative potassium channel cloned from mouse brain (Tempel et al., 1998, Nature 333, 837). A cDNA containing the complete open reading frame was isolated by ligation of partial clones. mRNA transcribed in vitro was microinjected into Xenopus laevis embryos. We measured membrane currents evoked by voltage clamp steps. A slowly activated outward current was evoked from a holding voltage of -90mV. This current had a maximum amplitude at about +40mV and was attenuated by TEA-aminopyridine and Ba2+ but was not attenuated by Cg3 (1mm). The reversal voltage of the current increased as a function of increasing K+. Thus, BRK1 encodes a potassium channel expressed in the retina, supported by NIM.


The role of taurine as a potential neurotransmitter in the retina has been questioned in numerous literature reports. One essential criterion for a neurotransmitter is the release of the substance from presynaptic nerve terminals. The present studies were designed to investigate the ten requirements on the spontaneous release of taurine and the effects of depolarizing agonists on the evoked release of taurine from a P, subcellular fraction of rat retina. The tissue preparation was loaded with [3H]taurine under conditions of high-affinity uptake. The absence of Ca2+ (+ 2 mm Ba2+) or Ca2+ (replaced by Br-) from the superfusion medium had no effect on spontaneous release. However, replacement of Na+ with choline caused a 50% reduction in the spontaneous efflux rate. Potassium (54 mM)-evoked release of taurine was unaffected by the absence of Ca2+ (+ 2 mm Ba2+). Veratridine (100 µM) was not as effective as K+ in stimulating [3H]taurine release as determined by measuring the areas (in arbitrary units) under the release curves (K+ = 14.4 units; veratridine = 1.9 units). Substitution of Li+ for K+ resulted in a much reduced evoked release of [3H]taurine (Li+/K+ = 2.2 units). The data suggest that the high-affinity uptake of [3H]taurine is into a pool of taurine that is non-vesicular. The results do not support the hypothesis that taurine is a neurotransmitter in the rat retina. (Supported by NHI grant EY07800).


In the vertebrate retina, a member of the kinase C (PKC) has been shown to label preferentially rod bipolar cells. Here, we examined in the rat retina the developmental pattern and the dynamic changes of cells expressing PKC-like immunoreactivity in the postnatal life of the normal adulthood. This was motivated in part by the possibility that the antibody might be used as a selective marker for examining the development of rod bipolar cells in the rat retina.

Faint PKC-LI could first be detected on postnatal day (PD)-10, being limited to the central region of the retina and labeling cell bodies located at the subretinal margin of the inner nuclear layer adjacent to the outer plexiform layer. On subsequent days, PKC-LI spread progressively toward the peripheral retina and in the axonal terminal bulbs of the retina. The plexiform layer began showing the first signs of immunoreactive labeling. By eye opening (PD-15), PKC-LI cells were identified, and their axons became immunoreactive. The axons traversed the entire vertical thickness of the IPL and divided into short branches before ending as terminal bulbs. By the end of the fourth postnatal week, the PKC-LI cells appeared mature, resembling bipolar cells found in adult. The morphology and the location of PKC-LI cells in both the developing and adult retina are consistent with those of rod bipolar cells. Rerouting to complete darkness from the day of birth resulted in a precocious expression of PKC-LI.

In summary, PKC is not expressed in immunohistochemically-detectable amounts until relatively late in retinal development and thus fails to be a useful marker for rod bipolar cells at the earliest stages of maturation. Our results indicate further that the timing and degree of PKC expression may be subject to regulation by light even before eye opening. Supported by PHS grants NS24830 and NS31340.


In rats, vitamin A deprivation lowers rhodopsin content, as determined by spectrophotometric measurements; however, both EM immunocytochemistry and freeze fracture analysis indicate that opsin density in the outer segments is unaffected by vitamin A deficiency (Katz, et al., Invest. Ophthalmol. Vis. Sci. Suppl. 31, 1990). The outer segments decrease somewhat in size but otherwise have relatively normal morphology. The Opsin/Opsin Heterogeneity Syndrome (OHS) is associated with age and adulthood lowers opsin sensitivity 100x (Stark et al. Naturwissenschaften 63, 513-518, 1976) by reducing both rhodopsin (spectrophotometry) & opsin (freeze fracture) (Harris et al. Nature 266, 486-489, 1977). On the other hand, rhodopsin recovery with replacement (EM immunolabeling & microspectrophotometry) (Sapp et al. Invest. Ophthalmol. Vis. Sci. 31, 1990) is labal high in rough endoplasmic reticulum (RER). Vitamin A specifically regulates opsin synthesis via transcription, translation or post-translational modifications in fly but not rat. This is logical from a photoreceptor-convertible rhodopsin 480 - metarhodopsin 580 system need only be synthesized once except for some daily turnover (Stark et al., J. Neurobiol. 17, 499-509, 1988); rod rhodopsin bleaches into opsin & chromophore, the latter recycled through supportive retinal pigment epithelial cells & reappplied to the opsin already present.
441.15

The b- and d-waves of the electrically eye-contracted (ERG) of the lizard Anolis carolinensis show a circadian rhythm CR in amplitude.

Removal of the lateral eye does not affect ERG amplitude or its CR. Also, the CR can still be phase-shifted by a new light-dark cycle.

Optic nerve section slightly decreases ERG amplitude and has complex effects on the ERG CR.

Removal of the pineal gland abolishes the ERG CR.

Application of melatonin to the isolated eyepepsid reduces ERG amplitude.

Surgical controls (sham optic nerve section, sham pinealectomy, lesions to the forebrain, and to the optic cortex) do not affect ERG amplitude or abolish the ERG CR.

An ERG CR is observed in some isolated eyepepsid.

These findings suggest that plasma levels of melatonin (which could be rendered non-periodic by pinealectomy) may carry circadian information to the retina. However, centrifugal signals in the optic nerve may also play a role, as might an oscillator within the eye.

Supported by NEI grant BRS-84644 (C. Karwoski) and a Ford Foundation Pre-Doctoral Fellowship (C. Collazo).

441.17


Melatonin has been reported to be a potent inhibitor of dopamine release in the rabbit retina (Dabbour, '83). We examined the effects of melatonin, dopamine agonists, and dopamine antagonists on ganglion cells, inner retinal neurons, and the ERG in a superfused eye preparation. In the outer retina melanin (5-50 μM) had little effect on core dopedinal horizontal cells, while dopamine, and its agonist SKF-38393 (1-5), and LY21776 (0.2) all caused a small depolarization of the outer horizontal cell resting potential. Curiously, dopamine antagonists SCH23390, had little effect on the horizontal cell light response, while metoclopramide (20-40 μM) caused a slight increase in the horizontal cell light response. At the level of the inner retinal neurons the actions of dopamine and melatonin were antagonistic. Dopamine depressed the light response of many on-dopedinatory units (including an identified photoreceptor), while melatonin increased them. Melatonin (5-50μM) increased the spontaneous firing rates of on-tropic ganglion cells, while dopamine depressed them. Melatonin decreased suprathreshold-off on-tropic ganglion cells, while dopamine increased them.

These studies support a role for melatonin inhibiting synaptic release by dopaminergic neurons. (Supported by EY06044 and P3E EYO6173)

441.18


A neural circuit has been identified in birds that may allow light to reflexively control chordal blood flow in the eye. The components of this circuit are: retina-suprachiasmatic nucleus (SCN)-medial Edinger-Westphal (mEW)-ciliary ganglion, choroid (Reiner et al., TINS, 1983). We have previously used laser Doppler techniques (using a TSI LASERFLO® Monitor) to show that electrical activation of mEW (Reiner and Fitzgerald, ARVO, '89) or SCN (Reiner et al., ARVO, '90) yields increases in chordal blood flow. In the present study we investigated whether this circuit mediates increases in chordal blood flow in response to retinal illumination in pigeons. Tissue overlying the superior pole of the eye was removed, the extracocular muscles curared, and the laser probe positioned against the sclera to measure chordal blood flow. Periodic ten second illumination of the retina of this eye, with an AO fiber optic light 2 cm from the eye, consistently yielded increases (20-100%) in chordal blood flow. Assessments of the occurrence of this light-mediated response in pigeons that had sustained complete unilateral destruction of mEW 3-4 weeks earlier indicated that destruction of mEW greatly attenuated this light-elicited increase in chordal blood flow.

Thus, the SCN-mEW circuit may be a neural substrate by which increases in retinal illumination yield increases in chordal blood flow. Effective control of chordal flow by this neural circuit could play an important role in maintaining a constant environment for retinal photoreceptors during light exposure. Supported by EY-05298 (A.R.)
442.1 ADAPTATION IN BULLFROG SACULAR HAIR CELLS INVOLVES AN ACTIVE MOTOR. A. Assad and D.P. Corey. Neuroscience Group, Howard Hughes Medical Institute; Dept. of Neurobiology, Massachusetts General Hospital; and Dept. of Neurobiology, Harvard Medical School, Boston, MA 02114

Bullfrog sacular hair cells adapt to maintained displacements of their stereociliary bundles in a manner suggesting a continuous adjustment of the tension applied to the transduction channels (Howard and Hudspeth, 1987; Hacohen et al., 1985; cf. Crawford et al., 1989). Calcium entry through transduction channels, which can be blocked by sufficient depolarization, appears to reduce the equilibrium tension (Assad et al., 1989). This model predicts that the bundle should pivot a small distance negatively when the cell is depolarized; such movements have been observed (Assad et al., 1989).

Correlation of these movements with the adaptation would imply that an intracellular motor molecule underlies the adaptation process.

We have examined further the relationship of active bundle movements to tension applied to the transduction channels (Howard and Hudspeth, 1987; Hacohen et al., 1985; cf. Crawford et al., 1989). Calcium entry through transduction channels, which can be blocked by sufficient depolarization, appears to reduce the equilibrium tension (Assad et al., 1989). This model predicts that the bundle should pivot a small distance negatively when the cell is depolarized; such movements have been observed (Assad et al., 1989).

Correlation of these movements with the adaptation would imply that an intracellular motor molecule underlies the adaptation process.

442.2 SINGLE-STEP PURIFICATION OF HAIR BUNDLES AND PRELIMINARY CHARACTERIZATION OF CONSTITUENT PROTEINS. P.G. Gillespie and A.J. Hudspeth. Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical School, Dallas, TX 75335-9036

Characterization of the molecular basis of mechanoelectrical transduction in hair cells has been hampered by difficulty in obtaining purified hair bundles, the principal components of which consist of hair bundle cells and at least one sensitive protein(s) of detecting extraordinarily small amounts of protein. We have therefore devised an efficient, "twist-off" procedure for purification of hair bundles and have developed a highly sensitive, nonradioactive means of protein detection.

We isolate hair bundles by gluing the basal surface of a saccule from the bullfrog (Rana catesbeiana) to the cover slip bottom of a perfusion chamber. After embedding the organ in low-melting-point agarose, we rotate the solidified agarose cylinder with respect to the immobilized saccule, shearing off the hair bundles at their relatively fragile bases. Light and transmission electron microscopy reveal few contaminants in the agarose plug containing the bundles. By the criteria of rhodamine-phallolidin labelling of F-actin, biotinylation of intracellular proteins, and exclusion of ruthenium red, over 90% of the stromelia resided after isolation.

We visualize the hair-bundle proteins by silver staining or by biotinylation followed by chemiluminescent detection, which allows the detection on electrophorets of as little as 50 fg (7 amol) of bovine serum albumin. Although actin constitutes over 75% of the protein in this preparation, or 10-20 ng per saccule, at least 30 other proteins can be resolved by SDS-Agar. Many of these proteins are exposed on the extracellular membrane surface, and several are extensively glycosylated; Triton X-114 extraction and phase partitioning suggests that some are integral membrane proteins.

442.3 OUTER HAIR CELL ELECTROMOTORITY: LOCALIZATION AND DISTRIBUTION OF THE FORCE-GENERATING MECHANISM. R. Hallowell, B.N. Evans*, and P. Zalis. Auditory Physiology Lab. (The Hugh Moore Center for Neuroscience and Psychology, Northwestern University, Evanston, IL 60201)

The location and mechanism of outer hair cell electromotority are currently unresolved. A series of experiments was conducted to distinguish between cortical (membrane-associated) and intracellular locations. Isolated OHCs were partially drawn into a microelectrode (Roth et al., 1990, in press) and voltage commands were applied across the cell.

Applied voltages resulted in anomalous movements of the cell's ends, that is, while one half contracted, the other half extended. This result strongly implies that the mechanism is associated with the cellular cortex, since the potential gradients across the membrane halves are also in signaltone, but the potential gradient within the cell is unidirectional (see figure). When the percentage insertion, p, of the cells was varied, the displacement of either end was described by a parabolic function, p(t).

These observations suggest that the displacement is the cumulative sum of the number of small movements driven in parallel. Measurements made at extreme percentage insertions deviated from the p(t) function, and were compatible with the assumption that the motile elements are restricted to the region between the cellular plate and the cell nucleus.

(Contributed by NIH grants DC0089, DC0708 and the Amer. Hear. Res. Found.)


The developing chick first responds to sound about embryonic day 11 (E11). Acoustic sensitivity and upper frequency limit increase with age, showing particularly marked improvement two days before hatching (E19). We have examined the electrical membrane properties of a cohort of selected groups of cochlear hair cells to determine what gated ionic currents might be acquired throughout this period. Hair cells were isolated from an area 0.8 to 1.2 mm from the apical tip of the adult cochlea, or from a proportionately located region of the smaller embryonic cochleas. Single hair cells were visualized with interference contrast optics on an inverted microscope and whole-cell, tight-seal recordings were made of membrane potential and transmembrane current.

Rapid, Ca-activated K current (IK(Ca)) underlies electrical tuning at frequencies greater than 100 Hz in hair cells originating 1 mm from the apical tip of the mature cochlea. A range of the cells from this cochlear region in the early embryo (E12-14) had a much slower, voltage-dependent K current, IK(VD), in combination with Ca current produced slowly sensitive (at less than 20 Hz) action potentials. The Ca current was similar in kinetics and magnitude to that found in mature hair cells. One week later (E19) significant amounts of IK(Ca) were found in hair cells from this cochlear region. High frequency (140 Hz) voltage oscillations could be evoked in current clamp. The acquisition of Ca-activated K channels by hair cells may be essential for functional maturation of the chick's cochlea.

442.5 DIFFERENTIAL RESPONSE OF ISOLATED INNER AND OUTER HAIR CELLS TO STIMULATION BY POTASSIUM AND CALCIUM. J. Schacht, D. Dalon* and G. Zalis* Kresge Hearing Research Institute, The University of Michigan, Ann Arbor MI 48109-0506.

Inner hair cells are generally considered the primary transducers in the mammalian cochlea while outer hair cells play a modulatory role in the transduction process. Motile properties are thought to be unique characteristics enabling outer hair cells to modify basilar membrane micromechanics in response to effective control. In isolated outer hair cells, increasing intracellular Ca** leads to reversible cortical contractions and cell elongation by a mechanism dependent on calmodulin and structural proteins. Depolarization by K* triggers mechanoelectric phenomena. A major question in determining the physiological correlates of such in-vitro responses is whether these phenomena are unique to outer hair cells.

Inner and outer hair cells were mechanically isolated from the guinea pig cochlea. Depolarization by KC* swelled both inner and outer hair cells by approximately 20% of their volume. The application of ionomycin, an ionophore permeabilizing the membrane to Ca**, increased intracellular Ca** levels in both inner and outer hair cells. This treatment. Movements were recorded in association with a decrease in the number of outer hair cells only and did not affect the shape of inner hair cells.

The results demonstrate that inner hair cells do not possess the mechanoelectric modulator that is found in outer hair cells. Thus a specific regulator of outer hair cell motility making this mechanism a most likely physiological modulator of a transduction feedback process. [Supported by NIH grant DC-00787]


Cochlear outer hair cells (OHCs) possess force generating abilities. Their rapid electromotority may be structurally related to the subsurface cisternae (SCs), a unique organelle composed of stacks of subplasmal smooth parallel membranes. Ototoxic doses of salicylate have been shown to reduce electroacoustic emissions in vivo as well as OHC turgidity and electromotility in vitro (cf. Brownell,W.E., Ear & Hear, 11:82-92, 1990). In vivo studies (Douch,E.E. et al. J. Laryngol. Otol., 97:793-799, 1983) have suggested that SCs are an important intracellular target for salicylate toxicity.

We have superfused isolated otocysts with media containing sodium salicylate (2mM, 5mM, 10mM for 10 - 60 min). Subsequently, the cells were fixed with an omically balanced glutaraldehyde solution and processed for transmission electron microscopy. OHCs maintained in standard culture media served as controls. The majority of cultured cells showed 1 - 13% preserved, smooth, unfenestrated SC layers. Suprasalicular lamellar or Hensen's bodies were only occasionally seen. Following salicylate exposure, the cisternal layers lost their parallel arrangement and became vesiculated and forming fenestrated cisterns of random orientation. There was also a more frequent occurrence of cytoplasmic membranous systems (probably Hensen body). The intermembranous cisternal distances appeared to be increased and in some instances distention of cisternal space was observed. These findings demonstrate a structural involvement of SCs in the ototoxic response to salicylate and strengthen the possibility that the SCs might be an important anatomical substrate for electromotility.

Supported by the Max Kade Foundation and Grants from ONR & NHI.

Sensory receptor cells (hair cells) of the chick's cochlea are classified as short or tall by the ratio of their apical surface diameter to length of the cell body. In short hair cells this ratio is greater than 1. Short hair cells are situated over the basilar membrane, in a position analogous to that of outer hair cells in mammals. Further, it is thought that the efferent nerve supply to the cochlea ends preferentially on short hair cells, as on outer hair cells, and that these axons release acetylcholine to produce their inhibitory effect. We have examined the effect of the cholinergic agonist, carbachol, on hair cell potentials selected from isolated regions of the chick's cochlea. Whole-cell, tight-seal recording was performed on cells that were exposed to a stream of 100 μM carbachol from a perfusion array.

In short cells carbachol produced a large hyperpolarization (as much as 30 mV from a resting potential of -40 mV). In voltage clamp carbachol produced an outward current that could exceed several hundred pA from a holding potential of -40 mV. Variation of the holding potential suggested that the current reversed near Eeq. The carbachol response desensitized over the course of several seconds. Responses to carbachol were observed in 17 of 18 cells. In contrast, carbachol had no effect on 18 tall cells from the same cochlear section, mimicking the proposed efferent innervation pattern.

442.8 VOLTAGE-GATED Ca2+ CURRENTS RECORDED IN VITRO FROM OUTER HAIR CELLS OF THE GUINEA PIG. X. LIN*, R.J. HUME, A.L. NUTTALL*, Kerge Hearing Research Institute* and the Dept. of Biology**, The University of Michigan, Ann Arbor, MI 48109.

Auditory efferent neural transmitter(s) may influence the transduction process in the outer hair cell by modulating voltage-gated ion channel(s) in the membrane of outer hair cells (OHCs). This hypothesis motivated our work on characterizing voltage-gated currents of the OHCs. Single outer hair cells from the guinea pig were voltage-clamped by whole cell voltage clamp techniques. OHCs were isolated enzymatically and placed in a modified pipette solution from the apical two turns of guinea pig cochlea in short-term culture. The pipette internal solution contained (mM): KC1 130, MgCl2 2, Hepes 12.5, glucose 10, pH 7.4. OHCs were cultered in a modified Hank's solution (NaCl 150, K4 MgCl2, 2, Hepes 12.5, glucose 10, pH 7.4). The external solution could be changed via a perfusion system. Currents (Leakage subtracted) in response to a series of depolarizing and hyperpolarizing voltage steps were recorded. A dot plot of responses to more positive test pulses were dominated by a delayed outward current with little inactivation. The outward current was completely suppressed by changing the medium in the recording chamber in a solution expected to suppress K+ and Na+ currents, which contained 4 mM CaCl2, 4, TEA-Cl, 100 mM 4-aminoipyridine (4-AP), 10 mM TTX 100 mM, Hepes 12.5 and Glucose 10. With this bathing solution, the inward currents recorded in response to the same test paradigm consisted of two components: (1) a fast-inactivating inward current first appeared at about -70mV, (2) a slow-inactivating steady inward current began around -30mV and peaked at about -20 to -30mV. The composition of the new external solution and the observation that the inward current didn't reverse for depolarizing potentials up to +80mV indicated that Ca2+ ions were the charge carriers that could not be present when the pipette internal solution was supplemented with 2 mM TEA and 1 mM 4-AP and currents in the bathed OHCs were inactivated. In conclusion, the data we have obtained so far support the idea that multiple types of voltage-gated Ca2+ channels as well as at least one class of K+ channels are present to mediate the inward OHC currents.

(Supported by NS05785)

442.9 WHOLE-CELL CURRENTS IN MAMMALIAN VESTIBULAR HAIR CELLS OF THE GERBIL. Stephen M. Edelman, Auditory Physiology Lab. and Dept. of Neurobiol. and Physiol., Northwestern University, Evanston, IL 60208.

In adult mammals, inner (IC) and outer (OHC) cochlear hair cells are contacted by afferent neurons which are morphologically distinct and completely segregated. IHC afferents are thick, myelinated neurons which project within the cochlear duct, produce few branches and typically innervate the nearest one or two receptors with punctate bouton endings. OHC afferents are thin unmyelinated fibers which extend far up to 600 μm before branching serially to innervate five to 50 receptors. To determine if vestibular sensory neurons of the cochlea innervate cochlear and vestibular hair cells we have recorded whole-cell currents of cochlear hair cell innervation are constructed I have examined early postnatal changes in the arborization of individual cochlear neurons in the Mongolian gerbil. Neurons were labelled through injections of Fluoresbend peroxidase into the apical turn of freshly excised cochlea which were subsequently minced and vitrified for three to four hours.

During the first few postnatal days (P0 to P2) some neurons had peripheral neurites which did not reach yet the IHC zone. These neurites were capped with growth cones of variable morphology depending upon their proximity to their hair cell targets. Other neurones had more complex arbors with neurites contacting both IHCs and OHCs. During the next two postnatal days (P3 and P4) neurones contacting both receptor types became increasingly rare. By P5, individual cochlear neurones contacted either IHCs or OHCs. At each postnatal stage the form displayed by an immature cochlear neurone de not affect its only input within the cochlear spiral; neurones located more apically generally possessed peripheral arbors which were less well developed. (Supported by NIDCD grant R29 DC00493).
443.1


The mammalian two types of hair cell (designated for the flask shaped II and for the cylindrical shaped I) differ in their morphology and synaptic connectivity. We have isolated both types from the guinea pig crista ampullaris to study the ionic differences in the membranes. It is convenient to further divide type II cells into tall (II) and short (III) sub-types. These have distinguishable ionic properties.

Using whole-cell patch recording (pipette containing KCI + BaPA), the mean zero current potential was -49.5±9.2mV (n=57), with no significant difference between cell classes. All cells showed a prominent outward K current which amounted to about 40%, 62% of type II, 31% of type I, and 17% of type I showed a current classified as an A current. A further component of the outward current was invariably reduced by 10 mM or 30 mM TEA (n=17), or by 200 µM Cd (n=12), indicating that all cell types have a K(Ca) current. Co-containing pipettes greatly reduced outward currents in type I and II cells, but left a small (300pA) inward transient which was blocked by 240 µM Cd. In type I and type I cells, the outward current was not reduced by internal Cs, suggesting an underlying K channel with a different selectivity.

At holding potentials more negative than -90 mV, type I cells showed outward rectification, eliminated by more positive holding potentials, presumably due to Ca loading. Such elevated intracellular levels of Ca activate a conductance with properties of a non-selective cation channel.

The results indicate significant differences in these two populations of cells. In particular the regulation of type I cell currents by internal Ca may determine the dynamic properties of the cell's response during labyrinth rotations.

Supported by the Wellcome Trust

443.2


Single photon computed tomography (SPECT) is used with various brain perfusion techniques to monitor the metabolic activity of the brain non-invasively. Increased metabolic activity can be secondary to increased neural activity which renders SPECT useful in monitoring brain activity during sensory stimulation. In this study SPECT images were generated in humans and anesthetized baboons when the hand was immersed in a noxious water bath (46-50°C) and compared to the hand immersed in neutral temperature water. Technical system: HMPAO was injected iv during stimulation, the head placed in a special head holder and the brain scanned with a 3 headed rotating SPECT scanner (TRIAD). SPECT and stimulus-control SPECT images were superimposed on congruent high resolution magnetic resonance images (MRI). The head holders supported fiducial markers filled with water and NCI and technetium-99m which were visible in SPECT and MRI images. Scaling and registration of SPECT images on MRI were done based on the markers on a SUN 4/330 computer running NMI software.

In baboon stimulus(50°C)-control(36°C) SPECT images, increased activity was seen in the contralateral posterior parietal cortex, frontal cortex and the ipsilateral cerebellum. Similar changes were seen in 2 other sessions in anesthetized baboons and macaques. In one human stimulus(46°C)-control(36°C) SPECT session no change was observed. This technique is currently being tested with higher stimulus temperatures in humans and using repeated measures in the baboons to establish statistical significance.

443.3

PSYCHOMETRIC AND SI NEUROMETRIC FUNCTIONS DURING ACUTE TOUCH OF GRATINGS IN MAN AND MONKEY. R. Sinclair and H. Burton. Department of Anatomy & Neurobiology Washington University School of Medicine, St. Louis, MO 63110.

Two M. mulatta and 4 human stroked fingertips over tips of horizontal gratings, and identified the smoother (smaller groove width). Grating ridge width was 250µm, groove width (GV) varied from 500 to 2000µm. Each grading pair had 1 of 4 standards (500, 1000, 1500, and 2000µm).

Humans discriminated GV differences of -10%, monkeys -20%. Neural Weber fractions, for area 3b and I cells with graded responses were computed as firing rate (FR) change between surfaces divided by AFR to standard. Neural Weber functions paralleled psychophysical ones. Threshold was high for comparisons against the 500µm standard (Weber's region), and comparatively low for other standards (Weber region). In many cells, AFR changes for equal GV differences were larger for rough than smooth surfaces, suggesting an exponential relationship between AFR and GV. Large neural Weber functions resulted for smooth surfaces, explaining skewed psychophysical Weber functions. Discrimination errors were associated with reduction in sensory information in some cells, due to changes in applied force or velocity of stroke.

Thus, cell responses in SI account for discrimination surfaces, and may form the basis for perception of textured surfaces.

443.4

NEURONAL RESPONSES IN SECOND SOMATOSENSORY CORTEX AND VPL OF MONKEYS DURING ACUTE TOUCH OF TEXTURED SURFACES. H. Burton, R. Sinclair and K. Nathian, Department of Anatomy & Neurobiology and McDonnell Center for Higher Brain Function, Washington University Sch. Med., St. Louis, MO. (Supported by NIDCD 00096)

Recordings were made from 62 SII and 20 VPL neurons in one M. mulatta trained to stroke its fingers across and discriminate between two gratings that differed in groove width (0.5-2.9mm). Preliminary results indicate that many SII cells showed little or suppressed activity during contact of their receptive fields (RFs) with gratings. These cells discharged bursts of impulses (1) on initial contact with the surface-bearing block, (2) when contact with the surfaces terminated and (3) when their RFs contacted an elevation between the two gratings on a block. SII cells with pacinian-like responses showed particularly prominent bursts in response to this elevation. Some of these cells showed increased, or positively or negatively graded with respect to groove width, and some had response functions that increased with the force of contact during stroking.

Responses obtained from VPL resembled some of those previously described in SI and, like peripheral receptors, showed combined effects of force, velocity and groove width. Relatively high firing rates were observed in some cells in VPL.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
443.5 EFFECTS OF A DORSAL SPINAL LESION ON TEMPORAL DISCRIMINATIONS AND ON PHYSIOLOGICAL RESPONSES OF PRIMATE S-I CORTEX. C.J. Vierck, K.W. White, A. Nakos, and E. Fridman. Dept. of Neuroscience, Univ. of Florida Col. of Medicine, Gainesville, FL 32610 and Dept. of Physiology, Univ. of North Carolina School of Medicine, Chapel Hill, NC 27514.

Interruption of the ipsilateral dorsal column (DC) in primates produced an area of tactile capacities (e.g., spatial localization on skin regions supplied by segments caudal to the lesion). However, lesioning produces a substantial decrease on a discrimination of different durations of tactile stimulation (i.e., 3 pulses at 10 Hz vs. longer trains at the same rate). In an effort to understand the neural mechanisms for this reduction of temporal resolution, single unit and evoked potential recordings from the primary somatosensory cortex (S-I) have been conducted in primates. Multiple unit and evoked potential recordings in the DC-fused region of S-I reveal activity that waxes and wanes. That is, stimulation does not reliably drive the cortical cells - especially at high firing rates. Supported by NS 07261 and DE07509

443.6 RESPONSIBILITY OF PRIMARY SOMATOSENSORY CORTICAL NEURONS TO VIBRATORY STIMULI DURING MOVEMENT VS. NO-MOVEMENT TASKS. B.J. Nelson and V.P. Douglas. Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, TN 38163.

The responses of some primate primary somatosensory cortical (SI) neurons to peripheral stimuli may be enhanced when these stimuli trigger movements as compared to when stimuli that make position should be maintained. It is unclear whether the responsiveness of other SI neurons would be suppressed in the same way and whether SI neuronal response enhancement and suppression differ depending upon a neuron's cortical location and an inhibitory or excitatory (RT) type. Three adult rhesus monkeys (Macaca mulatta) were trained to make wrist flexion and extension movements or maintain their wrist position following a delivery of a vibratory stimulus to the palm. Case was provided in accordance with the NIH Guide for Care and Use of Animals, revised 1985. The activity patterns were analyzed for 6 area 3a neurons, 13 area 3b neurons and 22 area 1 neurons. These neurons were selected because each had short latency (<60ms) vibratory related responses in both tasks, and each had a peripheral RT related to the hand or wrist. Neurons with cutaneous and deep RFs were treated separately. The magnitude of the vibratory stimulus-related activity was measured during movement trials. The vibratory response during no-movement trials was then measured over the same period. Area 3a, 3b, and 1 neurons with deep RFs exhibited a statistically significant (p<0.01) response enhancement during the movement task as compared with their vibratory response during the no-movement task. Area 3b neurons with cutaneous RFs also exhibited a (p<0.05) response enhancement during the movement task. Area 1 neurons with cutaneous RFs showed a significant suppression (<0.001) of their vibratory responsiveness during the movement task. These results suggest that an animal's preparedness to move in response to a vibratory stimulus is this movement tendency to modulate the sensory responsiveness of certain SI neurons. As changes in sensitive responsive SI neurons appear to differ depending upon the neuron's cortical location and RT type.

Supported by USAF Grant AFOSR 88-0179 to JNN.

443.7 A COMBINED MULTIELECTRODE-MICRODIALYSIS PROBE FOR USE IN THE MONKEY CORTEX. A.T. Kulcsar and B.A. Donzelli, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

We report here the development of a multielectrode/microdialysis probe which allows repeated penetrations over a long term into various cortical areas in awake, behaving monkeys. The microdialysis technique is used in conjunction with current multielectrode recording techniques (Cauller and Kulcsar, Exp. Brain Res., 1988). The microdialysis unit consists of an outer hollow-fiber semipermeable membrane and an inner flexible fused silica glass capillary tube. It is housed within a 25 ga hypodermic tubing which also contains 3 recording tips, spaced 400 μm for monitoring electrophysiologic activity. In one experiment, 20 min dialysate samples were collected before and after controlled cutaneous stimulation, and analyzed for amino acids using HPLC-ECD. In another experiment, bicuculline was infused directly through the probe which resulted in reversible disinhibition of the cortical network response to touch stimulation. These data demonstrate the utility of this probe design for the study of neurochemical and electrophysiological changes during localized drug delivery in the cortex of the awake monkey.

443.8 OSCILLATORY ACTIVITY OF SINGLE UNITS IN A SOMATOSENSORY CORTEX OF AN AWAKE MONKEY AND THEIR POSSIBLE ROLE IN TEXTURE ANALYSIS. E. Vaida and E. Abissar Dep. of physiology, The Hebrew University - Hadassah Medical school, Jerusalem 91010, Israel.

Neuronal activity was extracellularly recorded in the cortex of an awake monkey. Single units displaying oscillatory firing patterns were found in the upper bank of the lateral sulcus in a multi-modal area at the posterior border of SII. The majority of the neurons in the sampled area responded to tactile stimuli of the oscillatory activity. The neurons in a tri-modal fashion: 0-15 Hz, 15-50 Hz, 80-250 Hz. The most common frequencies were around 30 Hz. The oscillatory activity was not affected by analgesia, but was suppressed, in most cases, by tactile stimulation or self-initiated movements. Crosscorrelation analysis of all the neuron pairs (N=364) failed to detect signs of synchronization of the oscillatory activity of different neurons. Analysis of intrawave intervals in the spike trains of single neurons suggests that 65% of them were "intrinsically oscillators" while the rest were probably driven by external oscillatory source.

There are reasons to believe that the oscillatory neurons play a role in somatosensory processing: they are located in a somatosensory area; their firing rates and oscillatory components can be affected by tactile stimuli; and the bi-modal distribution of oscillating frequencies resembles the distribution of the three mechanoreceptor types (S,Aα,P) in the finger tip. The newly identified oscillators can play a key role in texture analysis if included in a Phase-Locked Loop (PLL) circuit, which measures an input frequency by comparing it to the frequency of a local oscillator. Such a model is presented in a companion abstract.

443.9 TACTILE TEXTURE DECODER MODEL BASED ON CORTICAL OSCILLATORS. E. Abissar and E. Vaida. Dep. of physiology, The Hebrew University - Hadassah Medical school, Jerusalem 91010, Israel.

The texture of a surface is determined by the density (spatial frequency) and the depth (amplitude) of its grooves and ridges. We propose a model that describes decoding of frequency modulation (FM) components of the texture. The heart of the model is a Phase-Locked Loop (PLL) circuit. This circuit, composed of a phase detector (PD) and a rate controlled oscillator (RCO), decodes FM signals and the provided that the input average ("carrier") frequency is within 4 times the intrinsic frequency of RCO. Many such PLLs are tuned to a specific frequency range, operate in parallel within a single "automatic velocity control" (AVC) loop. The role of the AVC loop is to maintain the input temporal frequency in a selected frequency range, by controlling the velocity of armpring movements. Thus, the model suggests that during surface exploration, the scanning velocity is first voluntarily set to generate sufficient temporal frequency, then the range of frequencies is fixed by the selected receptor modularity (SA, RA, or PD), and then the AVC controls the firing rate of the finger velocity.

A possible neural implementation of a single PLL is a corticothalamic loop, with three sets of neurons: A set in the thalamic ventralis nucleus serves as the PD and drives a set of inhibitory interneurons in the cortex, which in turn drives a set of RCO neurons feedback to the thalamic neurons. The capability of the thalamic neurons to detect phase differences is achieved by implementing an asymmetrical connection scheme in which the excitatory feedback synapses to the thalamic neurons, while the inhibitory connections to the excitatory synapses, which are multi-laminar (peripheral) input is mediated by conventional fast synapses. The surface frequency modulations are internally represented by the PD neurons population code. A computer simulation supports the feasibility of such implementation.


In previous studies we have shown that sensory transduction to single neurons in the somatosensory cortex and thalamus is modulated during movement and other behaviors. To define the mechanisms for this modulation we have used spike-triggered cross-correlation techniques to measure changes in the connectivity between neurons simultaneously recorded during these behaviors. Multiple single neuron recordings were obtained through a grid of 9-22 μm, microprobe electrodes implanted in the fissured areas of the SI cortex and thalamus in rats. Serial excitatory connections between neurons were indicated by narrow, short latency post-spikes that were delayed by 4-15 ms from the trigger spikes. Even with 20,000 trigger spikes, such peaks were found in only 10-20% of pairs of recorded neurons. Cortico-cortical (CC) and cortico-thalamic (CT) connections were found more commonly than CC-thalamo-cthalamic (T-C) or thalamo-thalamic (T-T). Briefly, the strength of these connections were found to be dynamically modulated during: 1) the refractory period of the post-synaptic neuron, 2) the conduction of two excitatory inputs on the same neuron, 3) stimulation of the peripheral receptive fields of these neurons, and 4) active locomotor limb movements. These modulations were not well correlated with changes in the background firing rate of the neurons. These results suggest that neuronal responses to spatiotemporal inputs during behavior are highly non-linear and state dependent, and may involve dendritic processing. Supported by PHS grants NS26722, AA06965, and AA00889, and AFOSR-90-0268 to JKC.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
443.11 EMERGENT PROPERTIES REVEALED IN MULTILAYER NEURAL NETWORK MODELS: FEEDFORWARD VS. FEEDBACK INHIBITION. J.P. Litz, M.A. Nicolelis, and J.K. Chapin, Dept. of Physiology, Duke University, PA 19120.

"Chaos" is an ubiquitous feature of non-linear systems producing such natural phenomena as fluid turbulence, population cycles, and variations in heart rate. The "quasi-periodic" behavior of such systems is often found to converge on a "strange attractor." The fact that the brain contains widespread non-linear inhibitory feedback circuits makes it possible for chaos to be stable. It is shown, however, that how chaos might be expressed in the discharge pattern of individual neurons in the brain, and how this influences the brain's overall function. This problem was addressed here by analyzing temporal spiking patterns of single units recorded in the cortex of rats during wakefulness and after pentobarbital anesthesia. To the contrary, the chaotic in spiking patterns, temporal memories of inter-spike intervals (ISIs) were visualized in 2- or 3-D sequential intervals. The temporal ISIs displayed features that tended to show relatively featureless clouds of raster points. After anesthesia SIFs from the same neurons exhibited attractor-like structures. To assess the dimensionalities of these SIFs, a standard technique was used in which lines were drawn on a log-log plot scattering the Euclidean distances between points (X-axis) against their correlation integral (Cd(y)), and the fractal characterization was added at each distance between each value on the X-axis). A line was calculated for each sequence of iterated "embedding dimension". The number of sequential intervals to show each point in an N-dimensional space. The dimensionalities of the spiking pattern was shown to be equal to the slope of these lines at the point they stopped increasing along with N. In these analyses, spiking activity from awake animals tended to yield dimensionalities of about 4.5 or greater, some being almost indistinguishable from random noise. By contrast, discharge of the same neurons during sleep or anesthesia had dimensionalities as low as 2.3. These results suggest that the anesthetized state may involve a marked reduction of the number of factors which normally influence cortical neuronal activity. Supported by NS32672, AO8985, AO8089, and AFOSR-89-0256.


Determination of stimulus location is a feature common to many sensory processes. It is assumed that the spatial acuity of such processing is determined by the size and density of receptive fields (RFs). For example, nociresponsive neurons in the medial thalamus have large RFs and are assumed to play no role in localization. In contrast, nociresponsive neurons in the lateral thalamus have small RFs and are implicated in localization. A computer simulation of a linear distributed network that locates point stimuli was developed. The network was composed of 1) an afferent input layer of "units" of density rho and radius lambda placed on a test surface of unit width and 2) two output "units" whose activity coded position of stimulus in cartesian coordinates. Connections strengths for the network were calculated using an algorithm which optimized point localization. We found that the error in the assignment of coordinates of point stimuli for trained networks varied inversely with the size of RFs (in contrast to the theoretical condition that large RFs can be associated with high spatial acuity. In addition we found that the profile of RFs had little influence on spatial acuity. These results indicate that high spatial acuity can be associated with large, overlapping RFs (in a distributed network). The roles currently assigned to medial and lateral thalamic nociresponsive neurons in localization is based on the assumption that the process (network) is non-distributed (labeled line model). The role, however, of nociresponsive neurons, with large and small RFs, in localization depends on the organization of the underlying network (degree of convergence of afferent input) which is unknown for these cells.
444.4 PREDICTIVE SACCADIC IN HUNTINGTON'S DISEASE (HD) J.R. Tian, D.S. Zee, S.E. Foltinstein, A.G. Lasker. Johns Hopkins University, Baltimore, MD 21205

Eye movements were recorded from 21 mildly-affected patients with HD and 20 age-matched controls. All patients with HD made significantly more saccadic types of errors than the controls.豆
A more than 30% of saccades were made incorrectly to the visual target. Patients also showed a defect in generating anticipatory saccades, to a target jumping in a predictable fashion. The antisaccade latency was 135ms in HD and 78ms in controls. The percentage of 21 patients had values > 2 standard deviations from mean. Mean percentage of anticipatory saccades (anticipatory latency < 100ms) was 28% in HD and 83% in controls. Three patients made no anticipatory saccades at all. Mean amplitude of anticipatory saccades was 14.2 deg in HD and 17.6 deg in controls. Eleven of 18 patients had values > 2 standard deviations from the mean of normal. We concluded that abnormal predictive saccadic tracking is common in early HD and that the basal ganglia probably play a role in generating predictive saccades.

444.6 POOR "SPATIAL" SIGNAL IN HUMAN SACCADE SYSTEM. R.S. Gordon and W.A. Fitcher. Dept. Clinical Neurosciences, Univ. of Calgary, Calgary, AB, Canada T2N 4N1

To make an accurate saccade to the spatial location of an object requires both visual information and a signal that identifies the position at the time that the image appears. To determine if the required eye position signal is available, the experimenter typically evokes an intervening eye movement shortly after presentation of a target. McKenzie and Lieberge (1986) argued that, in monkeys, the signal is available only if the intervening eye movement is saccadic. When pursuit movements intervened between target presentation and the saccade to that target, the saccades were specified by the visual signal; the intervening pursuit movement was ignored. We report that this dichotomy is absent in human subjects.

Subjects (N=4) tracked a moving horizontal (15-30°) that disappeared after 800-1200 ms. A spot was then flashed for 20 ms or less on the meridian, at horizontal offsets of 0-14° from the direction of gaze. Subjects were instructed to look at the spot where the background had flashed. When subjects looked toward this spot (after 200-300 ms) their saccades were compensated for 39% (range 17-55%) of intervening pursuit movement. In a double-step paradigm, where the second step occurred shortly before a saccade to the first target location, the screen was then blanked, performance improved, but remained poor (compensation 58%; range 43-74%). For intervening saccades or pursuit, compensation varied from 0-100% from trial to trial. Saccades to flashed target, where no movement intervened, were accurate (gain > 0.9 for most subjects).

These results suggest that the human saccadic system use eye position information regardless of whether it is generated by pursuit or saccades. This information is transmitted with low gain and high noise.

Supported by the Alberta Heritage Foundation for Medical Research


At the end of a saccade, the firing rate of primate motoneurons continues to decay even after the eye has reached its final position. This exponential decay, termed the post-saccadic slip, has a time constant Ts of approximately 30 ms (Goldberg, 1983). It has been hypothesized to compensate for a mechanical load element in the oculomotor plant; as such the slip is not specific to saccades. It should therefore change in eye position produced by other means. We examined neurons of the vestibular oculomotor system in the rabbit following eye movements evoked by changes in head position. Neurons were recorded in awake rabbits and identified as abducens motoneurons, interneuronss, or vestibular nucleus pretor motoneurons. RFs of the lateral rectus muscle, MDF, or oculomotor complex, respectively. Ramp-speed changes in eye position were produced by oscillating the animal's head about the vertical (0.5 sec ramp 8°/sec, 4 sec stationary, 0.5 sec -8°/sec, 4 sec stationary) in the light. Firing rate decayed exponentially during the stationary periods: a linear regression of the decay was used to determine basic discharge rate. The distribution of Ts was strongly positively skewed. Median Ts was 0.14 ms for abducens units and 1.7 sec (n=29) for the vestibular units. These values are 10-20 times larger than those seen in the monkey. In modeling the oculomotor plant of the monkey, a Ts term improves the prediction of firing rates for saccades of 20° in a direction opposite to the previous one (Fuchs & Blau, 1988). The long slide shown in the present study indicates that the influence of Ts extends to still lower frequencies in the rabbit.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
444.11

ON THE CENTRAL IMPLEMENTATION OF SACCADES IN LISTING'S PLANE. K. Hepp*, J. van Oostap, BJ.M Hess*, D. Straumann, V. Henn. Physics Dept, ETH, and Neurology Dept, University Hospital, CH-8091 Zürich, Switzerland.

We have investigated whether the central representation of saccadic eye movements in Listing's plane in the spatial (superior colliculus, SC) and temporal (rMLF) sacce
generator involves different types of firing or quaternary <ionts of final and initial eye position. The quotient model predicts how SC stimulate the area of Listing's plane, and that movement fields of saccade-re
lated burst neurons in the SC should have tor
sional components. With neuronal microstimulation at 60 sites in 4 colliculi of 2 monkeys with a 3D search coil we have found a clear violation of the quotient model. This was confirmed by a significantly better tor
sional modulation of burst neurons in the rMLF in difference coordinates. These fin-
dings suggest that Listing's law for visual saccades is implemented downstream of the spa
tial and temporal saccade generator.

Supported by ESFR (MUCON 3149; SNF 3199-
025239).

444.13

SACCADE-RELATED BURST CELLS OF THE SUPERIOR COLICU LUS ARE MODULATED BY ELECTRICAL STIMULATION OF ITS ROSTRAL POLE. D. M. Watizman, D. P. Munoz, L. M. Optican and R. H. Wurtz. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892, USA.

Recent experiments have shown a linear correlation between the decline in burst discharge and dynamic motor error in a neighborhood of saccade related burst neurons in the superior colliculus (SC) (Watizman et al., Expl. Brain Res., 72:649-52, 1988). To further study the relation between the discharge of these cells and eye movements, we interrupted saccades by microstimulation of the rostral pole of the superior colliculus in 2 pigeons with 2-6 pulse trains of 0.25-0.5ms duration of 10-15mA). 2-3 saccades were disrupted in 60 sites in 4 colliculi of 2 monkeys with a 3D search coil we have found a clear violation of the quotient model. This was confirmed by a significantly better to
sional modulation of burst neurons in the rMLF in difference coordinates. These fin-
dings suggest that Listing's law for visual saccades is implemented downstream of the spa
tial and temporal saccade generator. Supported by ESFR (MUCON 3149; SNF 3199-025239).

444.14


The notion of gaze position feedback is now widely used in all models of natural eye-head orienting movements. [1]. This idea was first an extension of the Zee et al. model of saccadic eye movements control, where an effector c
ponent of eye position was fed back and compared to desired eye position in order to control the saccade amplitude. Previous models always consider gaze position feedback as an additional input to the SC (superior colliculus), so that from the SC point of view, the generation of saccades is an open loop mechanism. In contrast, a recent model [2] assumes that the gaze control information is processed so that dynamic eye error is continuously updated within the SC itself. Here, we intend to present the comparison of gaze position versus gaze velocity feedback to the SC. The consequences of each feedback to the SC has been investigated in terms of: (a) Control accuracy (b) The different collicular networks supporting these strategies and their respective connectivity, and (c) Spatial and temporal transformation of eye movements into spatial-temporal signal. The net result of this study is the implementation and simulation of a collicular neural network performing both spatial-temporal and spatial-temporal spatial computations in a model that can be extended to a spatial-temporal case (for horizontal movements); this model, based on velocity feedback, achieves both spatial and temporal integration inside the SC, and is extremely efficient with regard to computational activity (energy).

444.15 SIGNALS RECORDED IN PRIMATE SUPERIOR COLLICULUS WITH SACCades EVOKED BY FRONtal EYE FIELD MICROSTIMULATION. R. Schlag-Rey, J. Schlag and P. Dassonville. UCLA, BRI and Dept. of Anatomy & Cell Biology, Los Angeles, CA 90024.

Projections from movement and visuomovement cells of the frontal eye field (FEF) to the intermediate layers of the superior colliculus (SC) have been demonstrated by antidromic stimulation (Seagroves and Goldberg, 1987). We sought to determine the nature of the FEF signal reaching SC movement cells in monkeys. First, movement fields of cell pairs (one FEF cell, one SC cell) were determined by unit recording during the performance of visual and saccade tasks. Then, the FEF site was stimulated (10-20 pulses of 0.2ms at 400 Hz, 5-30 μA) while recording from the SC cell. Preliminary results indicate that: (1) SC cells were excited by FEF stimulation when their preferred movement vectors were similar (movement field overlap). (2) Peak excitation appeared more related to saccade onset than stimulation onset. (3) However, excitation was still present, although weaker, at subthreshold intensities for evoking saccades. (4) FEF stimulation inhibited SC cells whose movement field did not include the vector of the evoked saccade. (5) The excitatory or inhibitory nature of the SC response depended on the site of stimulation in FEF, but not on the actual saccade vector when the latter was modified by the saccade collision paradigm. (USPHS grants EY02305 & EY05879 & NSF grant R01EY7-58034)

444.17 A DAMPED REPRESENTATION OF EYE POSITION IS USED IN OCULOMOTOR LOCALIZATION. P. Dassonville, J. Schlag and M. Schlag-Rey. UCLA, BRI and Dept. of Anatomy, Los Angeles, CA 90024.

Since the study by Hallett & Litts (1976) using a double-step paradigm of target presentation, it has generally been assumed that the oculomotor system is accurate in using a flash occurring immediately before, during or after an intervening saccade. Their findings differ from the many studies which show inaccurate perceptual localization of a flash occurring near the time of a saccade (e.g. Muhl 1976, Mauth 1976).

Honda (1989) has recently shown that the oculomotor system is not as accurate as compensating for intervening saccades as previously assumed. Data from our laboratory, using the colliding saccade paradigm, have also indicated oculomotor inaccuracies (Dassonville 1990), demonstrating that the artificial retinal error created by microstimulation of the primate frontal eye field is corrected to either a spatial error by adding an eye position signal that is a damped version of the actual saccade, or a motor error by subtracting a damped version of the change in eye position.

In the current study, two human subjects, in complete darkness, required to make a saccade to the location of a 2 ms flash (S2) occurring near the time of an initial, visually-evoked saccade to a previous 5 ms flash (S1). A monocular magnetic search coil was used to measure saccade accuracy. Under these conditions, the subjects might see S2 in a manner similar to that seen in Honda's and Mauth's perceptual studies. The former representation was found to be a damped version of the initial saccade (similar to that found with the colliding saccade paradigm), with a time constant of 50-120 ms. Flashes presented immediately before the initial saccade were localized in the direction of the saccade, while those presented immediately after were mislocalized in the opposite direction. Similar results were found when the initial saccade was self-initiated with S1 not present, removing any chance of an allocentric solution to the task. Methodological differences can be as least partly explain the dissimilarity of our results (as well as those of perceptual studies) and those of Hallett & Litts, whose task could be solved by the use of the allocentric relationship of the stimulii. (NSF grant R01EY7-58034 and USPHS grants EY05879 & EY02305)

444.16 INTRINSIC CIRCUITRY IN THE CAT SUPERIOR COLLICULUS IS HIGHLY DISTRIBUTED. M. Behan and P.P. Appel. Center for Neuroscience and Dept. Comparative Biosciences, University of Wisconsin, Madison, WI 53706.

There is a complex network of axonal arborizations and boutons labeled following small, localized injections of [H3]H-125 Tissue Culture and Biochemistry, University of Wisconsin, Madison, WI 53706.

In the present study, labeled boutons (with a 15-20 μm delay for force generation) were found to be distributed in the superficial layers of the superior colliculus. Labeled boutons are found in the entire field and represent the neuronal activity of the area centralis of the superior colliculus. The effects of this activity are a function of the superficial layers of the colliculus. However, there are certain differences in the distribution of labeled boutons in the deeper layers depending upon where in the superficial layers the injection is made. With a central field injection, there is a more dispersed distribution of labeled boutons in the intermediate and deep layers, with a distinct rostral pole. These differences in connectivity may provide the anatomical substrate for population coding of saccadic eye movements in the superior colliculus.


While increasing the inertial mass of the hand reduces the mechanical resonant frequency of the muscle-hand system (i.e., the physiological tremor frequency), the effect of mass on cyclic voluntary hand motion is unclear. Perceptual consequences also indicate that the muscle-hand system behaves as a lightly damped, second-order mechanical system, resulting perhaps from muscle-tendon elasticity, hand mass and joint viscosity. However, the muscle-hand frequency response for cyclic voluntary motion indicates third-order mechanics with break frequencies at about 1 Hz and 8 Hz. This suggests first-order mechanics (with a 15-20 μm delay for force generation) in the series with the lightly damped, muscle-tendon system. We studied the effect of mass loading on the frequency response (relating hand displacement and surface EMG envelope amplitude as a function of frequency) of the muscle-hand system for cyclic, self-paced, extension-flexion wrist motion. The frequency range studied was 1-10 Hz, with peak angular hand displacement at the fundamental (extended) frequency occurring between about 5-10 deg. The maximum load of 500 gm had a small but predictable effect on the amplitude-frequency plot, increasing the negative slope between 5-10 Hz. With 500 gm, the EMG-Displacement phase plot was shifted, with phase lag increasing by about 50 deg at 8-10 Hz. When considered in the time domain, the effect of mass on the kinematics of quick, point-to-point hand movements was small, and appeared to be mainly due to changes in the lightly damped, second-order sub-system.


Two multidirectional dynamometers were used to record the maximal isometric forces (MIF) exerted at the proximal phalanx of each thumb and to measure the forces developed during a simultaneous matching task of 10% MIF with the contralateral thumb. The isometric forces exerted by 12 normal female subjects (23 ± 1.5 years) with their dominant (D) and non-dominant (ND) sides were measured twice (2 sessions within a 2 week period) in 8 directions covering 360° by steps of 45° in the transverse plane of the longitudinal axis of the thumb. The MIF of largest magnitudes were obtained in the directions that brought the thumb towards the palm of the hand (flexion and adduction). No differences were found between the two recording sessions for these directions. There was no strength differences between the D and ND sides.

Force matching was also more precise in the more functional direction. The MIF with the non-dominant thumb force, angle and magnitude were both correlated with the errors in precision. However, errors in angle were different depending on the direction (largest in the abd./flex.). The precision of matching was similar for the D and ND sides. (Funded by the FRQS)
445.3 MODULATION IN PREHENSIVE FORCE WITH POSITIVE AND NEGATIVE LOAD FORCES. L.A. Jones1 and I.W. Hunt2. School of Physical and Occupational Therapy1 and Dept. Biomedical Engineering2, McGill University, 3654 Drummond St., Montreal, Quebec, H2W 1R7.

The forces used to grasp an object have been shown to vary linearly with the weight of the object, and the slope of this relation is inversely proportional to the frictional resistance of the hand. We tested the hypothesis that the slope of the hand friction being held (Westling & Johansson, Exp Brain Res 1984, 53:277-284).

The objective of the present experiment was to measure the modulation in prehensile force with positive (p) and negative (n) load forces. The forces were imposed on the hand and to examine how these pinch forces changed as a function of the frictional force between the skin and the manipulandum being grasped. Subjects held a manipulandum, which was composed of two symmetrically mounted disks, between the tips of their thumb and index finger. The manipulandum was a cylindrical electromagnetic linear motor that generated (under computer control) positive and negative forces. The position of the manipulandum was fed back to subjects on a visual display and they were required to maintain this constant while the forces generated by the motor increased from 0 to 30 N, in both the negative (p) and positive (n) directions. The forces produced by the motor and the fingers, and the position of the manipulandum were recorded on line by a MicroVAX computer during each 100 ms trial. Four different manipulandum surfaces, each with a different coefficient of friction, were used.

The changes in pinch force were tightly coupled to the forces generated by the motor in both the positive and negative directions, although for many subjects this modulation was asymmetric in that much greater pinch forces were generated to counteract negative load forces. As reported previously, pinch forces varied with surface structure, with larger forces being produced when the friction was smaller.


When subjects make rapid bimanual aiming movements over different distances, assimilation effects are shown. The long-distance limb usually undershoots its target, while the shorter-distance limb overshoots. However, in most of the studies showing assimilation effects, movement distance and end location have been confounded (i.e., the limbs move different distances to different target locations). In order to resolve this issue, 60 male and female students moved a light, aluminum lever in the sagittal plane with each hand to same or different target locations with either the same or different starting point. Assimilation effects were shown as overshooting in the left hand when either location or both distance and location were varied. The assimilation effects were reduced over 50 practice trials with knowledge of results (KR), but were also noted on a no-KR transfer phase of 25 trials. The results suggest that velocity or muscular force may be programmed by the motor system, rather than distance or location per se.

445.7 LEFT AND RIGHT HAND DIFFERENCES IN POINTING TO PERTURBED TARGETS. H. Carbahal and M.A. Goodale. Dept. of Psychology, Univ. of Western Ontario, London, Ontario, Canada, N6A 5C2.

The purpose of this study was to determine how rapidly subjects can amend movement trajectories in the left and right hands in response to unexpected target movement. On separate blocks of trials, subjects reached out and pointed with the left or right index finger to a target light that either remained in a central position, or was changed to a new location on the left or the right, upon release of the stimulus. Movement of the index finger were monitored by a WATSMART system.

There was no evidence for very early trajectory corrections (before peak velocity) for either hand, nor were there any hand differences in terminal error, RT, or time of correction. However, when the left hand was longer, peak velocity was lower, and time to peak velocity occurred later, when compared to the right hand. These findings also suggest that the target location. These results are discussed in terms of hemispheric specialization in motor control. (Supported by NRC grant MA-7269)


The role of tactile signals in regulating grasp force apparently is reflected in reports of excessive grasp force in patients with severe sensibility losses, and in the elderly, who typically show moderate sensibility impairments. However, the contribution of sensibility loss to excessive grasp force is not clear and requires experimental control of age, hand sensibility, and hand function. Moderate sensibility impairment was progressively induced in subjects who wore surgical gloves of increasing thickness. Some subjects underwent anesthetized nerve block of the thumb and index finger as well. Sensibility was measured using vibrotactile and two-point stimuli. With moderate sensibility losses, subjects exhibited two types of behaviors when lifting the test object: 1) excessive grasp forces, as hypothesized; 2) safety margins decreased substantially, apparently reflecting an impaired ability to monitor grasp forces; or 3) safety margins decreased or did not change, but subjects reduced the vertical acceleration of the object and limited the object's inertial load on the hand. Grasp force regulation is affected by even moderate sensibility impairments, but increased safety margins may reflect a consistently applied strategy only after chronic, rather than acute sensibility losses. Increased hand slipperiness may also contribute to developing this strategy of using excessive safety margins.


Typing is an ideal model system to study many aspects of skilled movement. It involves coordinated movement of the fingers of both hands. I is a serial process with a well-defined goal (the depression of a set of keys in sequence), but its execution can involve parallel processes (the movement associated with one keystroke can begin before the previous key has been struck). The movements can be measured with relative ease.

We have begun to study typing movements by asking subjects to type words in which all but one letter is typed with one hand, thus permitting us to define the prototypical movement associated with a single keystroke of the other hand. We characterize the movement in terms of linear translation and angular rotation of the wrist, the change in length of each of the fingers (measured from metacarpal joint to finger tip) and the angular orientation of the fingers. We find that each of these measurements is characterized by a unique pattern of movement (often involving all of the fingers of one hand). This pattern is independent of the movement of the other hand. Supported by USPHS Grant NS15018.

445.8 HAND DIFFERENCES IN PREHENSION: A KINEMATIC APPROACH. B. Sivak and C.L. MacKenzie. Canadian Centre for Habilitation and Research and Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

This study examined prehension differences between the hands. Right-handed subjects were instructed to reach for and grasp an object placed in front of them. Two factors were manipulated: 1) hand (left, right) and 2) type of grasp (collective, independent finger grasp). Analyses involved a quantitative examination of the kinematics of movement as related to transport and grasp components. With respect to the transport component, analyses revealed no differences between the hands. However, certain differences related to the type of grasp used. Peak velocity occurred later for a collective finger grasp than for an independent finger grasp regardless of the hand used. For the grasp component, subjects opened the hand wider with the left hand than the right hand for an independent finger grasp. There were no differences between the hands in the size of the hand opening when a collective grasp was used. The results suggest that hand differences may be related to the processing of visual-motor information subserving independent finger control.

(supported by NSERC Grant # OGP8303)
445.9

THREE-DIMENSIONAL (3D) ISOMETRIC FORCES EXERTED BY HUMAN SUBJECTS. J. T. Massey, J. T. Lurito, and A. P. Georgopoulos, Bard Laboratories of Neurophysiology, Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205. Human subjects exerted X-Y-Z forces on an isometric handle with the unclamped arm to move a cursor in the direction of a visual stimulus presented in an anaglyphic (3-D) display (Massey et al., J Neurosci Methods 26:123, 1988). Naive subjects were unable to perform the task with precision. Long term studies were undertaken with three subjects. The 3-D directional variability of the forces exerted and variance about the stimulus direction decreased with practice and reached an asymptote after approximately 4,000 trials; the half-angle at the apex of the 95% confidence cone for mean direction was approximately 10° at this time. Reaction times fluctuated widely during the earlier trials but attained relatively uniform values as skill increased. These findings suggest that a substantial perceptual-motor learning is involved in generating 3D isometric forces based on information derived from stereoscopic displays. (Supported by NSF BNS-8810642 and ONR N00014-88-K-0751.)

445.11

ACTIVITY PATTERNS OF ARM MUSCLES ASSOCIATED WITH RAPID WRIST MOVEMENT IN MAN. F. Aoki Department of Rehabilitation Research, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan. Adjustment of arm posture associated with rapid wrist movements was studied. Seven healthy adults, sitting and holding their right arm in mid-position at the shoulder and in 90° flexion at the elbow, were instructed to flex(f) or extend(e) the wrist as fast as possible. To examine whether the activity patterns of the upper arm muscles were related to the primate movement, the forearm was in two postures, pronated(P) and pronate(Pro). The surface EMGs of biceps brachii(Bi), brachialis(Br), brachioradialis(Br), and the prime mover, flexor carpi or extensor carpi were recorded to the angular displacement of the wrist. The sequences of the EMG onsets of the upper arm muscles were changed with the combination of the forearm posture and movement: Tr*Hi for Sup-E, Bi*Tr for Sup-F, Br*F for Pro-E and Tr*Br for Pro-F in all subjects except one in whom the pattern Sup-E was Bi*Tr. The durations from the onset of the earliest activity of the upper arm muscles to that of the prime mover were 4.5±0.3 ms(SD), 1.8±0.2 ms, 0.8±0.6 ms and 4.9±0.8 ms in Sup-E, Sup-F, Pro-E and Pro-F respectively. From these results, I conclude that the activity patterns of the upper arm muscles are related to the direction of the movement, and that the earliest activity of the upper arm muscles acts in the appropriate direction to coordinate the dynamic perturbation induced by the movement. And they are considered to be controlled by feed-forward mechanism.

445.12

HUMAN EYE, HEAD AND ARM ROTATIONS DURING REACHING AND GRASPING. P. Straumann, K. Nepp*, M. Nepp-Reymond, H. Sablatnig*, Neurology Dept., University Hospital, Dept. of Neurology and Brain Res Inst, CH-8091 Zurich, Switzerland. Using the search coil technique, we have measured simultaneously 3-dimensional eye, head and arm rotations in human subjects during various reaching and grasping tasks. Rotation axes and planes were all confined to Listing planes with small torsional components. For eye-in-space (=gaze) and head-in-space (=rotation) trials standard deviations were in the range of 0.5 - 1.5 deg, for rotations in the humero-scapular joint between 2.0 and 3.0 deg. The normal vectors for the gaze and head planes were directed frontally and almost in parallel, regardless of the task. The arm Listing plane depended on the reaching and grasping paradigm due to the distribution of the motion between the upper and lower arm. These data suggest that the neural network organizes all 3-dimensional rotatory systems in planes with fixed alignment, thus allowing linearization of synergies. Supported by NSF 3.501-0.86 and EMDO.

445.13

THE AXIS OF ROTATION OF THE ARM DURING POINTING. J. Hore, M. Goodale, and T. Vila, Dept. of Physiology and Psychology, University of Western Ontario, London, Ont., Canada N6A 5C1. The orientation of the arm in three dimensions was measured in normal human subjects to successive visual targets (max range 90°). A six foot diameter search coil system was used with a coil taped to the subject's wrist, such that the overall action of all joints was assessed. The rotations of the wrist from a central initial position form or prismatic plane surface were then compared to the 3-dimensional rotation space. This surface was similar to Listing's plane for the eye. (Tweed and Vila, Vision Res 30: 111-127, 1990) but was thicker, i.e., the torsional orientation of the wrist in space, was not the same. Two factors, contributing to the variability for a particular pointing direction, were a slow drift in the orientation adopted by subjects and a dependence on the location of the previous target. The planar surface represents the result of muscle mechanics because the subjects could be instructed to adopt a different wrist orientation which resulted in a shifted planar surface. The axis of arm rotation relative to space was fairly constant when pointing to successive targets. This axis shifted in space depending on the pointing direction of the arm. For example, in pointing from left to right a vertical axis was used which tilted when the arm was raised and tilted down when the arm was lowered, in both cases by approximately half the angle. Thus the rules of rotations for the arm when pointing appear to be similar to those used by the eye. Support by an MRC grant to J. Hore MT-6773.

445.14

A KINEMATIC ANALYSIS OF JOINT MOTION DURING CURVILINEAR POINTING MOVEMENTS. T. R. Kaminiski & A. M. Gentile. Teacher College, Columbia Univ., New York, NY 10027. The hypothesis investigated is that coordinated motion at the elbow and shoulder joints during both linear and curvilinear movements is based on a uniform strategy. Curved, point-to-point movements were performed by having the hand pass over an intermediate point between the initial position and the target. Results from 8 subjects revealed that the shape of the planar velocity profile remained essentially the same (smooth with one peak); even in instances where the hand velocity profile had multiple peaks. In contrast, elbow motion frequently demonstrated directional reversals and the velocity profile was dependent on the amplitude of displacement before and after passing the intermediate point. Inflections in the hand velocity profile occurred only when there was a large change in the difference in jotor displacement (shoulder vs. elbow) relative to the intermediate point. These results support the hypothesis that the joint control strategy is similar for both linear and curvilinear movements. In both cases, the shoulder provides a stable base for the organization of the movement. Furthermore, hand trajectories cannot be divided into multiple movement segments based on inflections in the hand velocity profile. These inflections may result from the mechanical interaction of the shoulder motion with the rest of the body. The results support the hypothesis that the joint control strategy is similar for both linear and curvilinear movements.

A further development of the equilibrium point hypothesis (λ model) is presented with the focus on the control of multi-muscle systems during goal-directed movements. According to this hypothesis, control is associated with changes of neurophysiological parameters (λs) which define the equilibrium state of the system. Muscle activations, forces and movement arise as a natural dynamic reaction of the system to the shift in the equilibrium. Changes in λ for a set of muscles can be coordinated directly by central commands or conditioned by the intermuscular interaction mediated by muscle afferents and interneurons. The afferent interaction is also under central control. In the λ model, multi-muscle commands are represented by vectors. Each vector is associated with a linear combination of λ parameters for a set of muscles and its length represents the strength of the corresponding command. In this space, there are basic vectors which represent functionally different types of coordination. For example, one vector command produces activation of muscles without changes in arm position while another may control motions about an individual joint without other joint motions. Any other control signals can be represented as a linear combination (superposition) of the basic control signals. Two versions of the model are presented, one for goal-directed arm movements and the other for mandible movements. Both versions reproduce characteristic kinematic and EMG patterns of natural movements.


It has been postulated that goal directed arm movements require an internal representation space to which both sensory and motor signals are referred. This interface implies the transfer of the position of the target's image in a body-centered system of reference. This operation takes into consideration the position of the eye in the orbit (DeJruf & at. Soci. Neurosci. Abstr., 1989). In the present set of experiments, the role of head position on the trunk has been investigated.

Human subjects sat facing a hemispheric screen, and were asked to move a hand-held pointer in the direction of LED's presented at several locations along the horizontal meridian. The trunk was not allowed to move. There were four initial conditions for eye and head: 1. eye and head aligned on the circle; 2. head on the midline, eye at 15°; 4. eye on the midline, head at 15°. In each of the four conditions, the subjects were either asked to orient their eyes towards the peripheral targets or to keep them in the initial position. The subjects oriented their forearm towards four peripheral targets (25 to 40°), in complete darkness.

In the eye mobile conditions, the analysis of the distribution of final arm position errors for the reference used is the trunk antero-posterior axis. In the eye immobile conditions, data suggest that a retinal frame of reference is used, though initial head position influences final arm position.

In conclusion, it appears that, in our conditions, the final position and not the amplitude of the pointing arm movement is controlled. This control operates within a body-centered frame of reference; but the final adjustments responsible for the final error rely on mechanistic frame that may differ, according to the available information about target, eye and head position.


It has long been known that rapid sequences of key-presses or spoken words exhibit a strong linear relation between the number of sequence elements and the reaction time to the beginning of execution. The goal of this study was to determine if there is a strong linear relation between reaction time and the number of elements of rapidly produced drawings. Normal right-handed subjects drew simple shapes (triangle, square, star) or made oscillatory motions (3, 4, 5 lines) according to standard reaction time methods. The number of peaks in the velocity profile of each drawing was used as the kinematical linear relation between velocity peaks and reaction time was found for the shapes, but not for the oscillatory motions. This finding suggests that oscillatory motions and line segments are primitive units of motor planning or behavior.

EQUILIBRIUM VECTORS UNDERLYING MOVEMENTS TO VISUALLY DISPLACED TARGETS. J.R. Flanagan, A.G. Feldman and D.J. Ostry. McGill University, Montreal, Quebec and Institute for Information Transmission Problems, Moscow, U.S.S.R.

The form of central commands underlying movements to visually displaced targets was examined within the framework of the equilibrium point (EP) hypothesis (λ model). According to this hypothesis, movement results from shifts from the equilibrium state of the metabolic system associated with the dynamic interaction of central commands, reflex mechanisms, muscle properties, and loads. Subjects produced pointing movements to targets located in a horizontal plane. The start of each movement was signaled by the illumination of an initial target. After a delay, the initial target was turned off and a second (displaced) target was illuminated. The position of the first target and the onset, amplitude, and direction of the displaced target were varied. The 3-D trajectory of the hand was recorded. Computer simulations based on the model were conducted to compare predicted trajectories, generated from theoretical central commands, with experimental trajectories. Previous reports have suggested that when a visual target is displaced, movement direction is continuously adjusted between the initial and final target (Sonderen, J.F. et al., Exp. Brain Res., 78:139, 1989). Moreover, it has been argued that this process may involve the superposition of the trajectories to the two targets (Henri, E. and Flash, T., Percept. 18, 495, 1980). We suggest that, at the planning level, central commands specify the direction and rate of shift (i.e., velocity vector) of the hand's equilibrium position. When the target is displaced, the equilibrium vector is simply shifted towards the new target.

IS FOREARM ORIENTATION PERCEIVED MORE ACCURATELY THAN ELBOW JOINT ANGLE? W. G. Darling. Dept. of Exercise Science, University of Iowa, Iowa City, IA 52242.

A number of studies have shown that the angle of the forearm in extraspatial personal space is perceived better in relation to axes external to the upper limb than in relation to the axis of the proximal arm segment. Exactly how such extrinsic angles are perceived has received little study. The perceptual task was simplified in previous studies by restricting either the angle of the arm segments or the plane of motion of the matching limb. In the present study subjects matched a remembered reference forearm or elbow angle (elbow flexion, forearm yaw or forearm elevation) with flexion or extension motion at the elbow only. The angle and plane of the arm segment during matching was different than in the reference position so that subjects could not simultaneously match the elbow and forearm angles. Elevation matching produced low constant and variable errors, supporting the theory that forearm angle is perceived accurately in relation to the gravitational axis. Matching of elbow angles produced larger variable errors and yaw matching produced larger constant errors. Subjects stated that elbow angles were easier to match because they used mental imaging of the forearm and its angle relative to imagined vertical and horizontal axes passing through the elbow to match the elevation and yaw angles. These results indicate that subjects use processes of mental imaging and rotation to perceive limb segment angles in extraspatial personal space.


Human arm movements when tracking visual targets are often intermittent, suggesting that an error deadzone is involved, i.e. a threshold that must be crossed before a corrective movement occurs. To test this, we have introduced an additional artificial error deadzone (AED) and measured the smallest size of AED which leads to impaired tracking performance.

Subjects tracked a pseudorandom waveform with a small hand-held joystick. The target was a stationary square in the centre of a computer screen. The joystick cursor was displaced horizontally from the target by the error between the joystick position and its desired position, as defined by the pseudorandom waveform (compensated tracking). In each trial, 10 of AED amplitudes between 0 and 20 pixels was introduced (0 - 1.26%). If the positional error was less than the current AED, then the cursor was displayed exactly on-target; at all other times its position reflected actual joystick position. We then computed the subject's integrated error score for the last 25s of each 30s trial, and plotted the average for each AED amplitude. Tracking performance varied considerably among subjects; smaller variance was seen between replications of the same AED and errors were approximately constant for each subject with small AEDs (≤ 6 pixels or 0.44°), but rose linearly above this threshold. These results indicate that the AED measured at the eye contributes to the intermittency of visually guided arm movements.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
445.21
FORCE REQUIREMENTS DETERMINE THE PATTERN OF ANISOMATIC MOVEMENT

There has been controversy regarding the factors producing height and/or duration modulation of the anisomatic burst during stepping. To examine this issue, subjects (21) were tested on the C. A. Bassile et al. Brain, Brain Sci. 12;189-230,89). We examined the agonist burst recorded from a wrist muscle (ECRL) of human subjects (n = 7) when they performed isometric movements at different load conditions. In each load condition, we compared the peak of the burst and its duration (at 25% of peak activity) for different types of movements (10% and 25% changes in the radial direction). The agonist burst for movements performed with a lightweight manipulandum was largely modulated in height (ave. change = 117%) and not duration (ave. change = 11%). In contrast, when subjects performed movements against elastic loads using a heavier manipulandum, modulation of burst duration became more prominent (ave. change = 60%) and modulation of burst height began to saturate (ave. change = 35%). In fact, the average duration of the agonist burst in one subject was as short as 51 msec for movements made with the lightweight manipulandum and as long as 211 msec for movements made in the loaded condition. Our results indicate that the force requirements of a task have a significant influence on the burst's height and/or duration modulation. Support: VA Med. Res. Service.

445.22

Reaching to grasp an object and lifting it requires a motion that must be transported to the desired location, and that functionally effective forces be applied to the object. The kinematics of the transport and grasp components until an object lift have been studied separately. However, it is important to determine whether both components are modulated in response to changes in the task. The current study examined the relationship between the two components (Jeanneaud, 1981, 1984; Martenik et al., 1987; Wing et al., 1986). Quite independently, the force patterns of the pinch grip after object lift have been studied in terms of movement parameters such as peak forces and their dependency on performance and control processes (Cole and Abbas, 1988; Johansson and Westling, 1984, 1997, 1988; Winslott and Abbas, 1989). Our purpose was to measure and compare kinematic and grip-force patterns prior to and after the application of functionally effective forces in a grasp, lift and replace task. Trials were blocked for three sets varying in weight (60,155,423 grams) but not visual appearance. The data was obtained for each task using a strain gauge which measured the vertical lifting force, and the horizontal gripping forces of the thumb and index finger. Analyses of markers placed on the thumb and index finger (grasp component), and wrist (transport component) revealed that differences in the kinematic patterns between hand lift and downlift reflected the application of forces after downlift contact. Temporal and kinematic analyses up to downlift contact revealed no differences; however, the time spent in contact with the dowel (prior to lifting), increased significantly as object weight increased. In addition, lifting and replacement forces varied systematically reflecting the need for stability during these two phases of movement. These results suggest a phase dependent coupling between the application of functionally effective forces and kinematic patterns. (Supported by NSERC)

445.23

Antipredatory postural adjustments (APA's) made prior to the initiation of multijoint arm movements may account for a variety of joint torques for smooth movement and successful goal accomplishment. This investigation demonstrates a relationship between multijoint arm movements and postural control. Two types of sagittal plane pointing movements, reaches (shoulder and elbow moving in the same direction) and whips (shoulder and elbow moving in opposite directions) were compared during three trials of each movement (3x3x4). Kinematic analysis revealed the whips to have longer movement times (X mt: 516 ms) and larger peak velocities (X Pv: 2900 m/s) than the reaches (X mt: 380 ms, X Pv: 1816 m/s). The APA latencies (time from change in shank acceleration to the initiation of wrist movement) were also different for the two types of movement. As appeared to time their upper limb movement with a peak in shank acceleration, suggesting an efficient relationship between body COM displacement and movement initiation. Comparison of body COM displacement to APA and wrist movement were also analyzed. It appears that the antipredatory postural adjustment varies with movement type, displacement and amount of force generated by the upper limb movement. The task constrains implicate differential control strategies which incorporate both the limb movement and the postural component.

445.24

We have previously reported that when subjects arm impulses isometric force to targets of unpredictable amplitude and direction in 2 dimensional space, these responses in a parallel and programmed by distinct mechanisms. Amplitude is programed as a continuous variable, direction as a discrete variable. We now determine whether similar distinctions apply to multijoint limb movements and assess the time needed to program these response features.

• Subjects moved a hand-aimed cursor on a digitizing tablet from a common start point; targets and a screen cursor were displayed on a computer monitor. A timed response paradigm was used: movements were initiated in synchrony with the last of a series of visual targets and targets appeared at unpredictable times before the last one. Within trial blocks, targets were at either of two distances and in either of two possible directions, target separations ranging from 15° to 60° on either side of the origin.

With widely separated (>60°) targets, default responses (<100 ms after target presentation) were directed towards one or the other target, for narrow separations they were directed towards the center. In contrast, extents were always clustered near the center of the range of required distances. As processing time increased (onset from 100 - 450 ms after target), the proportion of wrong direction responses decreased progressively, while the amplitudes and directions of initially biased responses were gradually adjusted. The processes responsible for the programming of movement direction therefore differ when alternative directions are widely separated or close together. Direction is processed categorically when directions are widely separated and as a continuous variable, like amplitude, when they are close together (Supported by NS2715).

445.25

We have previously shown that patients with large fiber sensory neuropathies make large errors in programming both the extent and direction of arm movements. These errors manifest as increases in variability and as biases in movement direction and extent. The purpose of this study was to determine the source of the biases. Two deafferentated patients and five normal controls moved a hand-held cursor from a central starting position to targets in 24 directions on a digitizing tablet. Target and cursor positions were sampled by a computer (X1, Y1) and values X2 and Y2. The hand and arm was blocked. The screen cursor was blanked during movement, to prevent visual corrections.

In normals, performance of the hand varies systematically with movement direction. This anisotropy reflects differences in the inertia being moved. Mean peak acceleration is twice as high in directions in which inertia is lowest. Normal subjects are able to compensate for these inertial differences by varying movement time, so that errors in extent show only a residual dependence on direction. The deafferentated subjects also show a strong dependence of peak acceleration on movement direction, but do not compensate the errors in extent show the same relative dependence on direction: as the variations in peak acceleration. Systematic biases in both initial and terminal direction are also present in normals but are not as consistent across subjects. These biases produce a clustering of responses in target directions and are markedly increased in deafferentated subjects. Both extent and direction biases in patients are substantially reduced by prior vision of the limb. We propose that the lack of control anisotropies in deafferentated patients results from an inadequate internal model of the mechanical properties of the limb. (Supported by NS22715).

445.26
INFLUENCE OF FLEXION AND EXTENSION FATIGUE ON SPEED OF MOVEMENT: N. J. Lambert, S. Peschke*, J. Draguns* and T. Hrubyjvi, Dept. of Sport Sciences/Biology, Univ. of Denver, Denver, CO 80208.

The influence of flexion (FLEX) and extension (EXT) fatigue on forearm flexion maximum speed of movement (SOM) was examined in 10 college-aged women. TEN SOM trials were performed pre and post to 3 bouts of dynamic fatigue at 60% of their peak flexion and extension strength, on each of 2 test days. Surface EMG burst patterns were recorded from mm. biceps and triceps brachii during SOM. Time to target increased following FLEX (11.5%, p <.05), but not following EXT fatigue. Duration of the biceps first EMG burst lengthened following FLEX (13%) and EXT (8%) fatigue (p <.05). The biceps to triceps bursts lengthened only following FLEX (23%) and EXT (18.5%) fatigue (p <.05). FLEX fatigue decreased the peak averaged EMG amplitude of the triceps burst (11%) and EXT decreased the amplitude of the biceps (22%) but not the triceps (2%) burst. The longer burst duration of the biceps did not compensate for the FLEX fatigue and resulted in a lengthened time to target. Following FLEX fatigue, the decrease was accompanied with a reduction of force to stop the slowed movement. Following EXT fatigue, reduced amplitude of the triceps did not result in the expected decreased time to target. A reduced amplitude of the non-fatigued biceps may have diminished the limb SOM. Thus, a factor other than the triceps counteractive force inhibited the biceps and restricted the flexion SOM following EXT fatigue.
446.1 A 40Hz Rhythm in Isolated Human Cortical Slices

A spontaneous 40Hz rhythm was recorded from human cortical slices removed during surgery for focal epilepsy. Signals were recorded extracellularly and subjected to a fast Fourier transform to a high pass filter. There was a consistent pattern of energy present in the 35-70Hz range. Previous activity in this range has been suggested to play a role in higher brain function. The activity was also subjected to a nonlinear dynamical analysis. Correlation dimensions were calculated and found to saturate at 6.7. The system could therefore be described by a low dimensionality deterministic chaotic attractor. This rhythm may represent repetitive activity in a two-neuron cortical feedback loop. Since it occurs in isolated cortical slices, it does not require connections with the thalamus or deeper structures and therefore does not represent activity in a thalamo-cortical relay circuit. All slices removed from epileptic foci also exhibited spontaneous synchronous seizure-like discharges during which this 40Hz rhythm could be detected.

446.2 MUSIC AS A TOOL IN ANALYSIS OF INTERSPRKE INTERVAL CODES FOR LANGUAGE AND MEMORY IN HUMAN TEMPORAL LOBE. D.E. Cathron, J. Rahn*, G.A. Clement, E. Lattou, P.F. Belt*, and D. Friel. Dept. Neurosurgery, School of Medicine, and Dept. of Physiology, Univ. of Washington, Seattle, WA 98195.

With informed consent and under institutional rules, we have commonly found modulation of firing frequency for normal human temporal lobe neurons by language and memory tasks, at nonosensory sites during epilepsy surgery. We had occasionally noted nearly identical cell (ISIs) or intercellular intervals (ICISs) in multimetric recordings, so we adopted a "stereotrode" (Cathron et al. J. Neurophysiol. 62(2) to separate individual cells from two multimetric units. Using the USIP Kernel compositional environment (Rahn, J. et al. Muscle 1(2): in press, 1990), we musically transformed the intercellular firing intervals of individual neurons, in which we saw orderly patterns, while slowing playback X10 from real-time to hear rapid sequences. The first transformation gave us a simultaneous presentation of both cells' notes playing till its next note. This allowed detection of repeating single and multiecell sequences, despite variation in tempo and melody. The second method used the reciprocals of each of a single cell's (instrument's) sequence of ISIs, scaling the resulting pitches into the 20 to 10,000 Hz range. This arrangement allowed identification of single cell ISI patterns, whether fixed or varying in tempo, better than of ICISs. The third method applied the second to all sequential intervals, whether ISIs or ICISs, to detect coding by ICISs more directly, as previously reported in patients but apparently without cell isolation (Bechtereva, N. et al. Brain Lang 1979; 7: 145-63), and in monkeys but without the ability to detect repeating sequences of differing tempo (Abel, D. and Gerstein, G. J. Neurophysiol. 1990; 63: 909-24). Samples of these encodings will be played and other mathematical music techniques shown for detecting more complex patterns. (Supported by NIH Grants N01274, N02482, and N17111 and a Horbach Award.)

446.3 THE CORTICAL CONSENSUS: A DISTRIBUTED DARWINIAN MODEL FOR RECOGNITION AND DECISION-TO-ACT. WILLIAM H. CALVIN. Univ. of Washington, NJ-15, Seattle 98195.

Small specialized regions of cortex exist but they are seldom essential. This is analogous to committees, which can function without all members present, where each expert member also sits on other unrelated committees, and committee size can change. MORTON KIRSCHNAUSS suggests that "When wide areas of the cortex are involved in a mental operation...[then] can be used either for a wide-ranging but shallow encoding, or for a single but difficult mental operation." But what neural mechanisms are "committee decisions" reached? Here I propose a model that builds on population-shaping from Darwinism, especially such runaway success as in speciation and the immune response. The Darwinian analogs are clearest when serial-order is involved: "get set" for hammering or throwing requires the same attention to proper ordering as does the genome's DNA sequence or the antibody's amino-acid sequence. Because ballistic movements are too brief for feedback to effectively guide them, one needs serial-buffer-like neural machinery comparable to the roll for a piano player, instead holding the activation patterns of the many different muscles of hand and arm. For accurate throwing, one requires many such arrangements in tandem (a "chorus of player pianos") to reduce timing jitter. In the Cortical Dynamics, I discussed the space-time applications of such serial machinery for shaping up strings of words; the "good-enough-to-speak" decision might occur when a population of randomly-varying candidate strings had (via differential reproduction of those rare highest by epistemic memories) evolved to near-cloons of one another (a "Darwin Machine"). Here I suggest that consensus also underlies higher-order recognition and that the many planning tracks greatly augment the associative memory needed for forming new categories, e.g., many different sequence populations coming up with a similar sequence ("apples-oranges-humans") constituting the recognition of a new category ("fruit"). Sequencing might play little role subsequently, only acting as scaffolding during concept formation.


"What is synchronized?" is the basic question in sensorimotor synchronization. We suggest that synchronization is given if the temporal central availability (TCA) of a sensory stimulus falls into the same time window as the predicted TCA of the feedback of a motor response (Pöppel et al. Naturwiss. 77, 1990, p. 89). In order to investigate this problem we have chosen when the subject has to follow a regular sequence of auditory (or visual) stimuli with motor responses. Normally, the subject has to anticipate the auditory stimuli by approx. 30 ms. Here we report that the interstimulus interval (ISI) determines the response mode. An important finding is that the ISIs up to 2 or 3 seconds. For longer ISIs subjects have to react to the stimuli; they can no longer be anticipated accurately. These results are interpreted on the basis of a temporal integration process whose capacity is limited to approx. 3 seconds (e.g., Pöppel: "Time Perception" Encycl. Neurosci., 1978). Supported by Deutsche Forschungsgemeinschaft.


Some examples are given of the logical status of theory in physics and biology, and of the limitations of the current science toward rates. The relationship between experimental data, principles derived from the data, and theories about the kind of theory modelled, and, in fact, attainable in neuroscience will be an abstract logical calculus (like the quantum mechanic calculus) which will make a synthesis of data and principles, as far as to explain in causal terms brain operation. A brief outline of such a theory will be developed using such useful principles as found in the tidy cell assembly, which work well in network theory, with distributed processing, and chaos theory. This framework will make use of a special theory of games, as well as heuristics including a neural conditioned reflex principle, random search and relative dominance among neural loops, and the formation of a global steady state condition in the CNS. Specific examples of possible neural substrates for some of these constructs (e.g., locus coeruleus and other thalamic nuclei as random search generators) will be proposed. The role such a theory might play in unifying data and principles in many cases will be discussed. Supported by the Mildred Andrews Fund.

446.6 DESCENDING INTERNEURONS IN THE SPINAL NETWORK OF THE GOLDFISH MAUTHER (M.) CELL ELECTROTONICALLY EXCITE PRIMARY MOTONEURONS. J.B. Fitch, Dept. Physiol., SUNY at Buffalo, NY 14214.

Morphological evidence suggests that descending interneurons excited by the M-cell terminate on many motoneurons, including the large primary motoneuron of the spinal network. The M-cell is known to play an important role in escape. To examine the synaptic connections between descending interneurons and primary motoneurons, we recorded intracellularly from a M-axon, a descending interneuron and a primary motoneuron (N=9). Firing of the M-cell led to firing of the interneuron and a multi-component EPSP in primary motoneurons. Direct activation of an interneuron produced a fast EPSP in the motoneuron that was very similar to, but smaller than, a component of the EPSP produced by M-cell firing. The latencies of the interneuron-motoneuron connections were between 2 and 3 ms, and EPSPs followed stimulation of the interneuron at more than 300 Hz. The short latency and fatigue resistance indicate that the interneurons electrotonically excite primary motoneurons. However, the data do not rule out the possibility, suggested by indirect evidence, that the interneurons also produce a late chemical input to motoneurons. Thus, primary motoneurons are excited by a combination of electrotonic inputs from descending interneurons and a previously described direct M-cell input. (Support: NIH NS05793)
CIRCUITRY

PROCESSING.


Some findings have been difficult to explain, as the occurrence of certain patterns may depend on the membrane properties of identified neurons. The connections we studied were those linking tegula afferents to wing elevator motoneurons. Octopamine did not significantly alter the afferent input, but it caused the appearance of a depolarizing waveform with a latency of 18-20 ms. This second depolarization is polypeptically caused by increased excitability of interneurons in the pathway from tegula afferents to motoneurons. Increased excitability was observed in some excitable interneurons presynaptic to elevator motoneurons. Short current pulses (5-10 ms, 0.5-1 nA) injected into these interneurons evoked burst like depolarizations which outlasted the pulses. The following findings suggest that these depolarizations are due to active membrane properties intrinsic to these interneurons; they were evoked in an all or nothing manner depending on the injected current and they could be terminated by slow hyperpolarizing current pulses. Furthermore long depolarizing current pulses (20 ms, 1-2 nA) evoked in some cases rhythmic bursting activity without activating the flight rhythm generator as demonstrated by recording simultaneously from other flight neurons. These preliminary studies suggest that octopamine modulates centrally the flight rhythm generator by inducing active membrane properties in a small population of flight interneurons. Similar effects of octopamine were observed in some neurons in the respiratory system.

FUNCTIONAL SIGNIFICANCE OF BI-THRESHOLD FIRING OF NEURONS.

D. C. Tam Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

The threshold for firing of action potentials for some neurons occurs not only at depolarized membrane potentials but also at hyperpolarized potentials. The bi-threshold phenomenon has been reported in a number of central neurons including thalamic (Bower & Llinás, J. Physiol. 278:205-226, 1984), inferior olive (Yarom & Llinás, J. Neurosci. 7:1166-1177, 1987), and hippocampal neurons (Staibath & Wilson, Soc. Neurosci. Abst. 15:339, 1989). Of particular interest is the fact that the intracortical potentials at hypodermic low-threshold spikes by Yarom & Llinás and 'baseline spikes' by Staibath & Wilson, which can be elicited naturally during the after-hyperpolarization (a.h.p.) following spike activation. The generation of the low-threshold spikes is a voltage- and time-dependent process, accompanied by anomalous rectification, occurs during a prolonged membrane hyperpolarization for de-activation of ionic conductances. The functional significance of the bi-threshold firing phenomenon was suggested to be involved in the two different rhythms generated by a neuron as a periodic bi-stable oscillator (Rose & Hindmarsh, P. Roy. Soc. London: Ser. B 225:143-159, 1984; Goldbeter & Moreau, J. Physiol. 95:277-287, 1984). It can be shown that bi-threshold firing may also be used for multiplexing signals by switching modes of operation in signal processing. Multiplexing can be considered as an opportunity to switch from a neuron rather than inhibition since under these hyperpolarized conditions the neuron actually becomes more excitable (i.e., closer to the low-threshold). The bi-threshold firing observed in the reported susceptibility to epileptic kindling at non-depolarized potentials in hippocampal neurons. It also suggests that Hebbian synapses may be strengthend not only by the depolarizing synaptic potentials but also by hyperpolarizing postsynaptic potentials. (Supported by ONR N00014-90-J-1355)

KINETICS OF JAW MOVEMENTS DURING DRINKING IN THE PIGEON.

R. Bermejo and H. P. Zeigler, Biopsychology Program, Hunter College (CUNY), New York, NY 10021.

In birds, as in mammals, drinking involves repetitive opening and closing movements of the jaw which vary the size of the oral aperture. However, in contrast to mammals, the few jaw muscles involved permit the movement of both maxilla and mandible. The kinematics of individual beak movements were studied during drinking in the pigeon.

Subjects were fixed in a stereotactic device using a skull-mounted head-holder and an infrared LED was attached to each beak. Drinking was elicited by immersion of the beak in water. Movements of the upper and lower beak were individually recorded with photoelectric light transducers and the analog signals were digitized (12 bit A/D; resolution 1 ms) for subsequent analysis.

The displacements of individual beaks were analyzed with respect to the cyrtical variations in the size of the oral aperture. Drinking in the pigeon involves a series of synchronized displacements of the upper and lower beak in the vertical plane, with the lower beak leading the mandible by 30-40 ms. In most cases, variations in mandible displacement accounted for most of the variation in gape amplitude. The data are consistent with previous behavioral and EMG studies of drinking in the pigeon.

(Supported by NSF Grant BNS88-10722 and NIMH Grant MH-08365)

ACTIVITY OF SPINAL NEURONS WITH DESCENDING AXONS DURING FICTIVE SCRATCH IN SPINAL TURTLES.


The turtle spinal cord contains sufficient neural circuitry to select and generate an appropriate form of the scratch reflex in a low-spinal, immobilized turtle, cutaneous stimulation in the neck and trunk regions evokes a rhythmic scratch motor pattern in hindlimb motor nerves; such stimulation in the pocket region elicits a pocket scratch motor pattern. We investigated the basis for scratch selection and generation by recording concurrent activity of hindlimb motor nerves and spinal neurons with axons that descend into the hindlimb enlargement.

This study utilized a spinal cord transection caudal to the forelimb enlargement and once within the hindlimb enlargement. Unit recordings were obtained via a microelectrode placed in the white matter at the caudal face of this multisegmental spinal spinal (2 cm cephalad to the cord transection) with a unipolar electrode. The electrode was inserted at a 45° angle, with the tip of the electrode pointed in the direction of the desired spinal level. The electrode was made of an insulated steel tip and had a diameter of 0.05 mm. The electrode was inserted at a 45° angle, with the tip of the electrode pointed in the direction of the desired spinal level. The electrode was made of an insulated steel tip and had a diameter of 0.05 mm. The electrode was inserted at a 45° angle, with the tip of the electrode pointed in the direction of the desired spinal level. The electrode was made of an insulated steel tip and had a diameter of 0.05 mm. The electrode was inserted at a 45° angle, with the tip of the electrode pointed in the direction of the desired spinal level.
446.15 CLASSIFICATION OF NOISE ACTIVITY POTENTIALS (APs) BY MEANS OF A NEURAL NETWORK EMPLOYING BACK-PROPAGATION. J. Espinosa E. and J. Quiza T.*, C. Wernick, J. Beamer., Faculty of Sciences, UNAM, México 04510.

Metal electrodes allow to simultaneously register APs from different neurons, creating the need of techniques for spike sorting. As a first step we have been implementing a method to classify APs. Using a database of 64 significantly different APs as the training set, we generated a network with 97 processors forming two separate layers of 64 and an output layer with 7 neurons. The network is modifiable at will. APs consist of 128 points normalized between 0 and 1. These values go into an input layer that receives them unchanged. For employing the back-propagation training algorithm we set the initial weights (990) between -0.5 and 0.5. After 35,000 iterations that took 20 min CPU in the TITAN(Ardent), with 16 MFLOPS, the total system error was less than 0.01. The final weights are fed to a conventional PC for classifying APs. Gaussian noise was added to original APs randomly modifying amplitude up to 60%. The network was able to classify 90% of them correctly. The next step will be to sort APs embedded in a noisy spike train.


Features of two potassium conductances implicated in the acquisition of conditional reflexes: slow activating (gK+) and gK(-), were incorporated into a 6*6 element artificial neural network with real-time, thresholded spike transmission and adaptive learning. Two of these models, incorporating different adaptation algorithms changed gK(A) in proportion to the product of this current and an EPSP-induced second messenger concentration, and changed gK(-) as a function of a spike-induced second messenger-concentration. This network acquired two distinct representations in response to presentation of stimuli: one resulted in the maintenance of a selectivity to forward pairing vs. simultaneous or backward pairing; the other was sensitive to contiguous pairings of stimuli. The acquisition of one representation did not markedly interfere with acquisition of the other; this network may accordingly serve as an example of a system which minimizes the postulated inherent cross-talk between functionally dissimilar representations (see Minsky & Papert, 1968).


A major difficulty in developing neural network simulations of biological data has been the absence of techniques for interweaving neurobiological data and connectionist architectures. Techniques for implementing such networks are being developed and tested in our laboratory. The present developments permit experimental neural data to be directly incorporated within connectionist architectures. The spatial distribution of cell bodies, the physiological properties of each cell, and the stochastic history of the cell's spiking trains were retained. Signum activation functions describe the behavior of each unit. These functions were directly derived from the cell's spike history data. Each neuron was thereby represented by a unique network unit that mimicked both the mean firing frequency and temporal dynamic range of the physiologic cell. An "analog" function that preserved both the exact spiking times and the relative spiking tendency of each neuron was substituted for each spike train. The "effective" influence of each cell is reflected in the cellular dynamic range and the stochastic temporal modulations of individual cell firing patterns. These types of networks provide a means of accurately representing the biological data, while preserving cell discharge characteristics for other accessible parameters may be altered. Functional hypotheses can be developed to compare experimental and analytical issues related to the biological nervous system. Alternatively, hypotheses derived from the analyses of experimental neural data may be tested by appropriate modifications of the synthetic architecture.

446.18 MEMORY Properties in the 1000-Neuron Inhibitory-Feedback Network Model for Simulation of Neocortical Circuits. C.D. Myer and D.J. Woodward. Dept. of Cell Biology and Neuroscience, UT Southwestern, Dallas, Texas 75235.

This laboratory has reported previously on the capability for information storage, or memory, in the states of networks of mutually-inhibitory model neurons. Continuing investigations of this memory in this Network Model (which construction was inspired by medium spiny neurons) has revealed several new properties showing how state maintenance and state switching occur. The studies described here were performed using the Simulation System (Neurosi. Abstracts 1989, #14618). The calculations were performed on a Sun SPARCStation 370 computer. The 1000-Neuron Network Model is made up of 1000 model neurons with realistic electrical properties, with parameters set by the user. Each neuron is connected to a variable, spherical, domain of nearby neurons by inhibitory synapses. Excitatory inputs via excitatory synaptic activity are controlled by the user, and Gaussian "noise" (representing lumped, random synaptic activity) may also provide excitatory and inhibitory effects. The parameter settings in initial experiments were such that, after decay of inhibition and potassium conductances for a neuron, the membrane potential reached threshold, and the neuron fired. When done using the 3x3 stimulation System (Neurosi. Abstracts 1989, #14618). The results showed: 1) the memory network requires setting parameters so that PSP's are relatively long-lasting; 2) even though states could be approached with high fidelity, they are only a few ms, only a few super-suppressed excitatory impulses; 3) state changes with local synaptic modifications do not necessarily produce globally, despite the high connectivity. Also, powerful global noise can induce spontaneous network activity which facilitates evolution to a system state ground state. This modeling suggests that the structural organization of neurons and their interactions which supports memories. Support from the Texas Adv. Res. Proj., Biol. Humanities Found., MH44337, DA02338, and AFOSR 90-146.
ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONSHIPS


The organization of the mediodorsal thalamic nucleus (MD) of the cynomolgous monkey was examined with retrograde and anterograde axonal tracers, and an analysis of cyto- and myeloarchitecture. Our results verify the conclusions of previous studies (e.g., Goldman-Rakic and Porrino, '85), but also demonstrate additional features of the organization of MD. In addition to the magnocellular division of MD (MDm) and its projection to orbital cortex, we recognize a separate caudal division as the source of projections to medial prefrontal cortex. Projections of MD were studied by placing small injections of fluorescent retrograde tracers restricted to single cytoarchitectonic areas in the medial or orbital prefrontal cortex, or the agranular insula. After injections into orbital cortex (areas 11, 13 and 15), labeled cells are found in MDm, which largely corresponds to the densely myelinated MD pars fibrosa in myelin preparations. In contrast, injections of retrograde tracers into medial prefrontal cortex (areas 32, 24) label cells in MDm which are associated with the dorsal and caudal limits of MDm; cells projecting to area 24 are found at the dorsal edge of MDm, along its entire rostralcaudal extent, while cells projecting to area 24 are concentrated in the caudomedial portion of the nucleus. These parts of MD partially correspond to MD pars densocellularis of Olczewski (‘53), but more precisely correspond to a poorly myelinated portion of MD referred to as pars caudalis in human material (Hassler, ‘59). Cells projecting to the agranular insula are limited to the medial edge of MD, a region which is poorly myelinated and may correspond to a medial extension of the caudodorsal division of MD. Agranular label from prefrontal cortical regions (areas 32, 13, and agranular insula) reciprocates this pattern. Support: NIH DC0095-20.
447.5 SYNAPTIC ORGANIZATION OF CORTICAL EFERENTS AND GABA-CONTAINING SYNAPTIC ELEMENTS IN THE MEDIODORSAL THALAMIC NUCLEUS OF THE MONKEY. E.A. Brodal, D. DeKloet, N. Dyar, J. H. Goldstein, and N. Rapin. Sect. of Neuroanatomy, Yale Univ. Sch. of Medicine, New Haven, CT 06510

We examined the synaptic organization of cortical inputs and GABA-containing synaptic circuitry in the nucleus mediodorsalis (MD) of the monkey. For each MD, we injected an additional monkey using GABA immunohistochemistry (counterstain). Quantitative analysis of the density of silver grains over different regions of the MD. The synaptic organization of cortical inputs and GABA-containing synaptic circuitry in the MD was similar in all monkeys examined. In four additional monkeys using GABA immunohistochemistry (counterstain).


Higher-order motor areas, implicated in initiation and execution of skilled movements, have been localized on the medial surface of the frontal lobe in both monkey and human. SMA, the dorsal portion of area 6, has connections with primary motor cortex as well as somatosensory cortex and the spinal cord. Each is thought to have a somatotopic representation of the body surface. Additional premotor areas have been suggested in individuals with epilepsy (e.g., He et al., 1989). We have investigated the connections of prefrontal cortex with the SMA and cingulate using small injections of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) in each of several distinct areas of prefrontal cortex (Walker, 1940).

Analysis of WGA-HRP - labeled axons in the SMA, particularly in areas Ba 9, 12, and 11/3 have connections with rostral SMA. All of these areas are also connected with the anterior third of the cingulate gyrus and in some cases with the ventral bank of the cingulate sulcus. In one additional area (Ba 46, 10) the rim of the principal sulcus (46) is also connected with cingulate cortex. Another subset of prefrontal areas (Ba 12, 5, 11/3) were connected with the middle third of cingulate cortex, including the ventral bank of the cingulate sulcus in some cases. A few areas (Ba 46, 10), 12, and 11/3 have connections with the SMA (45) project on the SMA and/or cingulate cortex in ways which appear to be topographic manner. These data could provide transcortical pathways by which prefrontal cortex influences the motor cortex to initiate a particular movement...
447.11
POSTNATAL DEVELOPMENTAL CHANGES IN PARVALBUMIN (PV) IMMUNOREACTIVE (IR) AXON TERMINALS OF BASKET AND CHANDLER NEURONS IN MONKEY NEOCORTEX. M. Akil and D.A. Lee. Department of Psychiatry and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213.
Basket and chandelier cells are GABAergic inhibitory interneurons, and important regulators of pyramidal cell function. We used immunohistochemical methods to determine the location of PV, a calcium binding protein, in axon terminals of these two classes of interneurons during postnatal development in monkeys. Neocortex. We examined prefrontal area 46, primary motor area 4 and visual areas 17 and 18 cortices of Macaca mulatta and fascicularis monkeys aged 4 days to adult. PV+ varicose perineuronal baskets were prominent as early as 4 days of age and absent in the adult. Both the time course and pattern of their disappearance were region-specific. No PV+ baskets were seen in area 46 at any age. Chandelier neuron axon terminals were formed by PV+ varicocities aligned in vertical rod-like structures. PV+ IR fibers appeared later than perineurial baskets and remained present in all age adults. The differential regional, laminar and developmental pattern of PV+ IR axon terminals of chandelier and basket interneurons may provide insight into their function.

447.13
CORTICAL AND THALAMIC CONNECTIONS OF THE ORBITAL CORTEX IN THE RAT. F. Comblé and F. Crételin. CNRS URA 1121, Bât. 440, Université Paris-XI, 91405- Orsay, France.
In the orbital cortex (OC) of the rat, Zilles's atlas [1985] reports 3 subdivisions: the medio-ventral (MOV), lateral (LO) and ventro-lateral (VLO) parts of OC, from which connections are virtually unknown. The retrograde transport of fluorescent tracers diaminido yellow and true blue was used to investigate the connections of these orbital areas with the other areas of the medial frontal cortex (MFC), the postgenual cingulate (CG) and the retrosplenial (RS) cortices. In contrast to the adjacent insular and prelimbic cortices, OC receives direct afferents from the central part of the ipsilateral mediodorsal nucleus of the thalamus, the anterior olfactory nucleus and the pyriform cortex. These results strongly support the hypothesis of an homology between OC in rat and OC in monkeys. From the pattern of afferents from OC to MFC, postgenual CG and RS, another delineation of the subdivisions of OC in 2 parts appears: 1) a rostral orbital area, including MOV and lateral part of LO and VLO, which projects to MFC, postgenual CG and RS, and 2) a caudal orbital area, including the caudal part of LO and VLO, which projects only to anterior cingulate and prelimbic cortices. Supported by HFSP grant.

447.15
CYTOARCHITECTURE AND CORTICAL AFFERENTS OF THE MONKEY ORBITOFRONTAL CORTEX. Morecraft, R.J., Geula, C., Schatz, C., and Mesulam, M.-M., Harvard U., Boston, MA.
Orbitofrontal cortex (OCF) is a major paralimbic component of the primate brain and can be divided into agranular (perifornical/paracentral), dysgranular (bicortical) sectors. The agranular and dysgranular sectors are more caudal and medial than the granular sector. HRP injections were made within the agranular/dysgranular sector of the OCF in one monkey and into the granular sector in another. Both cases contained labeled neurons within the cingulate cortex and all components of the prefrontal cortex. The case with the tracer injection in the agranular/dysgranular sector of OCF contained labeled neurons in the perirhinal parietal operculum, high order association cortex of the superior temporal sulcus and the granular sector of the parietal operculum. The case with the tracer injection in the granular sector of OCF showed a different pattern. Labeling was seen in the agranular/dysgranular components of the insula and temporopolar cortex. The case with the tracer injection in the agranular/dysgranular sector of OCF showed a different pattern. Labeling was seen in the agranular/dysgranular components of the insula and temporopolar cortex and there was less extensive labeling in the superior temporal sulcus. In the amygdala, labeling was much more intense in the case with the agranular/dysgranular injection. The observations show that the monkey OCF displays a complex pattern of connectivity which follows cytoarchitectonic lines of demarcation and which is in keeping with its behavioral affiliations.

447.12
HETEROSTEREOGENEITY OF THALAMIC CELLS PROJECTING TO LAYER I IN POSTERIOR PARITIAL CORTEX OF CAT AND MONKEY. E. Avendaño, J. Stephane* E. Reinoso,* and F. Reinoso Subiyay. Department of Morphology, Medical Faculty, Autonomous University, 28029 Madrid, Spain.
The thalamic neurons projecting to the superficial layers of cortical areas 5 (cat and monkey) and 7 (cat) were investigated by using superficial deposits of retrograde tracers in one hemisphere. The injections in homotopic locations of the contralateral one. In the cat, labeled neurons, found in the IP-Pul complex, and in paralaminar nuclei, were fewer in number and smaller in size in cases of superficial deposits than in cases of deep injections. In non-superficial portions of IV, however, there were no size differences. In the monkey, similar differences were noted in the caudal division of IV and in Lp and Pul, whereas no such differences were found between neurons labeled in the dorsal and medial divisions of IV, and in the ventral posterior inferior nucleus. The intralaminar and midline nuclei exhibited retrogradely labeled neurons only when deep layers were injected. These findings led to the point of a widely distributed layer I-projecting system (LIPS) of neurons which is not most nuclei are interspersed among neurons projecting mainly to middle or deep layers. The paralaminar nuclei, which would be a part of this system would provide through their projections to layer I in the posterior parietal and frontal cortices regions, which may provide for recruiting responses and spindling activities. Supported by Grant P87-0130 from CICYT.

447.14
REACTIONS TO FAMILIAR OR NOVEL ENVIRONMENTAL CUES AT ORIENTATION BEHAVIOR OF RATS WITH UNILATERAL OR BILATERAL MEDIAL AGRAURAL CORTEX (AMG) LESIONS. J.M. Vargo, F.J. Best, L.O. Wallis, S. Lopez, and J.L. Corwin. Department of Psychology, University of New Orleans, New Orleans, LA 70148.
To examine the role of dorso medial prefrontal cortex attentional processes, Long Evans rats were trained to traverse two straight alleys for food. Subjects received sham, unilateral, or bilateral, AMG lesion. Postoperatively, the subjects received further training. Then the goal boxes were changed such that they contained novel cues or contained familiar cues not previously experienced there. Alley running latencies of the bilateral indicated that they acted the unfamiliar (p < .01) as well as familiar cue change. These results are similar to those found with bilateral medial dorsal thalamic lesion (Stokes, K.A. & Best, F.J. Neuroscience, 13:1067, 1987). Unilateral AMG operated demonstrated no deficits in the alley. The subjects also underwent orientation testing in a visual, tactile, or auditory stimuli presented to each box side. Bilateral operated did not demonstrate orientic deficits while left AMG operated demonstrated contralateral neglect (J.M. Vargo et al., Exp. Neurol., 102:199, 1986). Even though bilateral lesions fail to produce neglect they do produce spatial context recognition deficits.

447.16
SOME SPECIES DIFFERENCES OF THE CHOLINERGIC BASAL FOREBRAIN IN RAT AND MONKEY. Ch.R. Schatz, C. Geula, R. Morecraft and M.-M. Mesulam, Harvard U., Boston, MA.
We investigated NADPH-diaphorase activity and calbindin D-28k immunoreactivity (Carrerasa generously provided by L.B. Hersh, B. Wainer, M. Celio) in the cholinergic projection neurons of the rat and monkey basal forebrain (Ch4-5, and brainstem, Ch6-7), specifically comparing species differences. We could confirm the nearly total overlap between NADPH-diaphorase activity and choline acetyltransferase (CHAT) immunoreactivity in the brainstem(neuromas and Ch4-5) in both species. In the rat, we found NADPH-diaphorase activity in up to half of the CHAT-positive cells of the vertical limb nucleus of Broca's (Ch2) and in approximately 20% of CHAT-positive cells of the medial septal nucleus (Ch1), but not within the nucleus basalis (Ch6). In the monkey basal forebrain (Ch4-5), colocalization of NADPH-diaphorase activity and CHAT immunoreactivity occurred very rarely, if at all. As reported previously by Celio and Norman (Anat. Embryol., 1983), the nucleus basalis (Ch4) neurons in the monkey contain only CHAT immunoreactivity, suggesting that only ChAT and calbindin are colocalized in these neurons. No such overlap could be found in the rat. Adjacent sections stained for CHAT and calbindin revealed CHAT immunoreactivity in Ch3 and 6 in both the monkey and the rat. These findings demonstrate a major species difference in the chemoanatomical profile of the cholinergic basal forebrain in rodents and primates. The cholinergic projection cells of the brainstem did not show such species differences.
447.17


The ultrastuctural morphology of neurons containing choline acetyltransferase (ChAT) and their relative to catecholaminergic terminals exhibiting immunoreactivity for the catecholamine-synthesizing enzyme tyrosine hydroxylase (TH) were examined in the rat medial septal, diagonal band nuclei using dual immunohistochemical and peroxidase anti-peroxidase labeling methods to simultaneously localize antibodies from two different species. Perikarya with ChAT-immunoreactivity (ChAT-I) were large (20-30 μm), elongated and contained an abundant cytoplasm. Many of the perikarya and dendrites with ChAT-I were surrounded by glial processes. Synaptic junctions on ChAT-labeled perikarya and dendrites were both symmetric and asymmetric with 68% (135 out of 197) of the presynaptic terminals unlabelled. The remaining terminals were immunoreactive for TH (25%) or ChAT (7%). Of the synapses formed by ChAT-labeled terminals, 31% (55/176) were with unlabeled perikarya and dendrites; whereas 7% were with perikarya and dendrites with ChAT-I. Also, 21% of the ChAT-labeled terminals contacted the same unlabeled perikarya and dendrite as a TH-containing terminal or were in apposition to TH-labeled terminals (25%). The remaining terminals with ChAT-I were either in apposition to unlabeled or ChAT-labeled terminals or lacked associates. These findings provide cellular substrates for direct synaptic modulation of (1) cholinergic neurons by both catecholaminergic and acetylcholine and (2) non-cholinergic neurons by acetylcholine alone or in conjunction with catecholamines. (Supported by grants MH42834 and HL18974.)

447.18


Choline supplementation during embryonic days (ED) 12-17 and later during postnatal days (PD) 16-30 – but not PD 0-15 – was shown to produce long-lasting facilitation of spatial memory processes in the adult rat. We now report that these two time frames represent anatomically and morphologically distinct phases of choline related influence. Should sphere analysis of dendritic trees from Golgi stained sections using a 3-D Euteic Neuronal Tracing System revealed that all three perinatal choline treatment/shift of the function relating dendritic intersections/ring to sphere radius from the cell body for granule cells in the dentate gyrus. In adult rats (8 months) distributions of dendritic intersections/ring initially centered at a sphere radius of 88 ± 11 μm from the cell body for the controls were shifted to a sphere radius of 38 ± 6 μm with no accompanying change in the overall number of dendritic intersections for perinatally choline treated rats. In contrast, only the dendritic trees of pyramidal cells in the CA 3 region of the hippocampus for rats in the ED 5-23 group were affected, whereas, the dendritic branching of pyramidal cells in the lateral posterior and lateral dorsal nuclei of the thalamus was shifted leftward for subjects in the PD 0-15 group. These differential patterns of sensitivity exhibited by neurons in the hippocampus and thalamus during early development can be profitably related to the differences in memory facilitated exhibited by the timing of choline supplementation.

HIPPOCAMPUS AND AMYGDALA: NEUROPHYSIOLOGY II

448.1


Opiate receptor agonists are thought to excite hippocampal pyramidal neurons through inhibition of GABAergic inhibitory interneurons. We have previously shown that both μ and δ selective opiate agonists produce marked increases in population spike amplitude when applied to superfused hippocampal slices. However, no study has directly compared the actions of these agonists on measures of interneuron inhibition. We compared the effects of the μ selective agonist DAGO and the δ selective agonist DPDPE on IPSPs recorded intracellularly in CA1 pyramidal cells using antidromic (recurrent IPSPs) and subthreshold, orthodromic stimulation (feedforward IPSPs). While the μ agonist DAGO (100 nM) resulted in a significant 50 ± 3% (n = 10) reduction in feedforward IPSPs and a significant 48 ± 7% (n = 5) decrease in recurrent IPSPs, the δ receptor agonist DPDPE did not reduce either type of IPSP (n = 8 and 6, respectively), at a concentration 5x higher than that which marked the population spikes (500 μM). Neither DPDPE or DAGO had any effects upon resting membrane potential, input impedance, or afterhyperpolarizations. The possibility that DPDPE was not acting through the reduction of inhibition was explored by applying DPDPE or DAGO to hippocampal slices following pretreatment with the GABA-A antagonist bicuculline methiodide (BMI; 10 μM). The excitatory effects of both DPDPE and DAGO on population spike responses were blocked with BMI. These results suggest that although μ and δ receptor activation mediate diminished GABAergic inhibition in the hippocampus, these receptors do so via different mechanisms.

Supported by NIH grant DA 07670 and the Veterans Administration Medical Research Service.

448.2

IMAGING OF INTRINSIC OPTICAL SIGNALS IN HIPPOCAMAL SLICES DURING SYNAPTIC ACTIVATION. D. Hochman and B.A. MacVicar, Neuroscience Research Group, University of Calgary, Calgary, Alberta T2N4N1.

Neuronal activity has been shown to induce changes in intrinsic optical properties of CNS tissue. We have used dual-color video microscopy to examine whether there are changes in transmittance of light through hippocampal slices during synaptic activity. Synaptic responses from Schaffer collateral stimulation were recorded simultaneously with the acquisition of video images of the CA1 region. Images acquired during stimulation were subtracted from control images. Repetitive synaptic activity was correlated with a progressive increase of light transmission through the stratum radiatum which recovered to control levels several sec after stimulation was terminated. Blocking synaptic transmission with kynurenate (1.5 mM) or 0 Ca++-EGTA perfused blocked the optical changes indicating that post synaptic activation was necessary for the signals. Furusoeim (5mM), an anion transport inhibitor, reversibly blocked the synthetically- evoked optical changes. Transient changes in optical properties of the slice may represent cellular swelling and be correlated with volume changes in the extracellular space. Supported by MRC (Canada).

448.3

COMPARTMENTAL MODELLING OF THE SPACE CLAMP PROPERTIES OF CA1 HIPPOCAMAL PYRAMIDAL CELLS. E.W. Stockley* and H.V. Wheal, Department of Neurophysiology, University of Southampton, Bassett Crescent East, Southampton, SO9 3TU, U.K.

The technique of recording synaptic currents utilising whole cell patch is gaining widespread interest. Nevertheless, the space clamp effectiveness of the method may be limited in highly branched neurons such as pyramidal cells. We have simulated the performance of a realistic voltage clamp in a compartmental model based on a serially reconstructed CA1 pyramidal cell (Wheal,H.V & Stockley,E.W, Soc.for Neurosci.15:403, 1989).

Synaptic inputs to the model were simulated using either transient (alpha function) conductances or current pulses. The resulting synaptic and somatic currents as well as the voltage profile in the dendrites were calculated for inputs at different positions in the dendritic tree. The voltage deviation from the clamp voltage, and somatic current were used as a measure of clamp effectiveness.

Results demonstrate the poor clamp effectiveness for inputs at motor cortex. We have also presented the results from this model with those obtained using branched and collapsed equivalent circuit models.

Supported by the Wellcome Trust.

448.4

BICUCULLINE AND PHACOLONE IONTOPHORESIS BLOCK AMYGDALAR PHYSIOLOGICAL RESPONSES TO BASAL FOREBRAIN STIMULATION IN RATS: AN IN VIVO STUDY. L. E. Mello, A. M. Tan* and D. M. Finch, Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024.

Amygdaloid responses to electrical stimulation of the basal forebrain were recorded in vivo from chlorided anesthetized adult male Sprague-Dawley rats. Extracellular recordings were performed using 5-25 MG microelectrodes filled with 3 M NaCl saturated with Fast Green, attached to a multibarreled micropipet for iontophoresis.

Intracellular recordings showed that responses usually consisted of antidromic activation followed by IPSPs, and EPSPs. Inversion of evoked IPSPs using KCl pipes indicated that the IPSPs were at least partly mediated by chloride influx. Iontophoretic application of bicuculline could induce burst firing that was restricted to the first part of the evoked IPSP, whereas effective application of phacolone blocked only the last portion of the IPSP. These results were observed for cells in every tested amygdaloid nucleus. Histology showed stimulating electrodes to be in the diagonal band, ventral pallidum, olfactory tubercle, bed nucleus of stria terminalis and nucleus accumbens. In conclusion, the results suggest that inhibition produced by basal forebrain action on the amygdala is at least partly mediated by GABAergic mechanisms. Supported by NIH Grant NS 23074 and FAPESP (Brazil).
448.5 A DIGITAL NEURONAL SPIKE DETECTION AND CLASSIFICATION SYSTEM USING ACTIVITY AND MOBILITY WAVEFORM DESCRIPTIONS. J. D. Bronzino, R. J. Austin-LaFrance and P. J. Morgan. Dept. of Engineering and Computer Science, Trinity College, Hartford, CT 06106.

We have developed a digital neuronal spike detector and classification system utilizing the Hjorth waveform descriptors activity (power) and mobility (slope spread) (A&M) to classify neuronal activity derived from multi-unit recordings. The system represents a significant advance in both the speed and accuracy with which spike-like events are separated and classified by a real-time computer-based system. The system was tested against the reduced feature matching technique of peak-to-peak amplitude and duration in order to illustrate the versatility of this measure. Multi-unit recordings from hippocampal region CA1 and the dentate granule cell layer were used to compare the ability of A&M measures to effectively separate two distinct cell groups recorded during the behavioral states of REM and slow-wave sleep. Classification of spikes was achieved by using feature histograms and cluster plots generated from the A&M measures. The results of these comparisons indicate that A&M measures can be calculated in a significantly faster speed and provide better characterization of spike than conventional approaches to real-time spike classification. (Supported by NIH Grant # R1NS24135-01A1)

448.7 OSCILLATORY PROPERTIES OF THE HIPPOCAMPAL FORMATION BLOCKED BY A REVERSIBLE BLOCKADE OF THE MEDIAL SEPTUM. L. V. Coen, S. Nazel-Caudrillaud, B. H. Blend. University of Calgary, Department of Psychology, Behavioral Neuroscience Research Group, Calgary, Alberta T2N 1N4

The intrinsic neural oscillatory (N-O) property of the diagonal band of Broca (vDB) region is critical for the appearance of theta (6) field activity in the hippocampal formation of the intact animal. If field activity can be generated in the hippocampal formation by systems, intraseptal and intrahippocampal infusions of the cholinergic agonist, carbachol. Previous work from our laboratory demonstrated that a "like" field potential can be generated in hippocampal slices perfused with carbachol. We were therefore interested in determining if microinjection of carbachol in the hippocampal formation of the intact urethane-anesthetized rat could produce field activity during a reversible procholine HCl blockade of the vDB. The MS/vDB blockade was confirmed by electrical stimulation of the dorsomedial-posterior hypothalamic nucleus. Experiments revealed that intrahippocampal infusions of carbachol failed to produce field activity when the MS/vDB was blocked in this manner. However, when microinjection of carbachol was followed by a microinjection of bicucullina (a GABA-A antagonist) in the same hippocampal site, a "like" oscillation similar to that seen in hippocampal slices was observed. The oscillations were 3-6 Hz with frequencies ranging between 5-12 Hz and had a depth profile similar to that of 4 Hz, with a phase reversal in stratum radiatum. The extent to which these oscillations share the same mechanisms as 6 Hz activity, is currently under investigation.


Optical recordings with voltage-sensitive dyes has become a powerful tool for investigating neural preparations in which use of multi-electrode approaches would be difficult. The laser scanning microscope employing optical methods has been used as a tool for investigating a variety of in vivo preparations (see: Ann Rev Physiol, 51, 1989; Ann Rev Neurosci, 11, 1985), due to the multiple technical difficulties encountered with this technique. The new scanning microscope system we have developed is based on a laser, an acousto-optical deflection system, a specially designed inverted-type epifluorescence microscope as well as a computer-controlled scanning unit. The prototype version of this system is capable of recording from up to 100 sites at a rate of 20 frames per second, thus enabling the monitoring of fast events such as synaptic and action potentials. For a first step he system has been employed for multisite monitoring of neural activity in hippocampal brain slice preparations (300-500um). After placing the slices in a recording chamber, a bipolar electrode for stimulation was positioned in the Schaffer collaterals while an extracellular microelectrode was used for recording from the CA1 region (Saggau et al., Neurosci Lett 69, 1986). After staining the preparation with a voltage-sensitive dye (25um RH-414 for 30min) the recording system was set up by positioning the optical recording sites under visual control. When using a 40x objective lens the spot size is 10um and the maximally scanned area 150um x 150um. Due to the noise characteristics of A&M measures to noise ratio simultaneous monitoring of neural activity of many sites was possible. The extension of the described system to confocal microscopy with its improved spatial resolution of all three dimensions is under development. (Supported by a grant of the Heidenhain Foundation to P. Saggau)

448.9 ENHANCEMENT OF DENTATE GYRUS FIELD POTENTIALS THROUGH GLUTAMATE-INDUCED ACTIVATION OF THE SUPRAMAMMARY NUCLEUS (SUM) IN RATS. G. P. Lavoie and C. W. Harla. Dept. of Psychology, Memorial University, N. John's, Newfoundland, Canada A1C 5S7.

A large number of cells centered in the lateral aspect of the SUM innervate the supragranular layer of the dentate gyms and send diffuse fibers to Ammon's Horn of the hippocampus. Recently, it has been demonstrated that electrical preactivation of the SUM enhances pentothal-dentate gyms evoked field potentials (Mizumori et al. J. Neurophysiol, 61:15-31, 1989).

Considering the large number of fibers that pass through this region, we investigated the effects glumataminic stimulation of this region had on dentate gyms field potentials in urethane-anesthetized female Sprague Dawley rats. The pentothal bath was stimulated with a bipolar electrode at a rate of 100 shocks (6.25 V), evoking an EPSP and a population spike in the dentate gyms granule cell layer. L-glutamate (500 mM, typically 100-300nM) was delivered by either pressure injection through a glass micropipette or through a 3 gauge cannula, directed to the SUM. The latency to the start and peak of the spike, and three measures of spike size: height from first positive potential to the spike peak, height from the peak to a tangent, and area under the tangent. Mean values for six events were compared to 95% confidence intervals based on the control period (10 min.). Glutamate injection in the area of the SUM associated with hippocampal activity consistently produced an enhancement of the population spike reflected in all 3 measures of spike size. The spike height (spike start to spike peak), averaged over 1 min., was significantly enhanced from 123 to 161% of the control mean. The period of the enhancement ranged from 2 to above 30 min. No consistent effects were found on EPSP slope or the latency measures. Sites just outside of the hippocampal projection area did not produce spike facilitation.


The integrity of the medial septo-hippocampal limb of the diagonal band nucleus (MS/vDB) is essential for the generation of hippocampal theta (6) rhythm. Previous results from this laboratory indicated that theta oscillations in hippocampal cells are generated by acetylcholine, and we have speculated that off cells are inhibited by GABA. In order to assess these possibilities, we examined the responses of off- and on-cells to reversible suppression of the MS/vDB induced by proprine HCl (P). Rats were anesthetized with urethane and implanted with hippocampal reference and hypothalamic stimulating electrodes, as well as cannulae in their MS/vDBs. Once a cell was isolated and baseline recordings obtained, P was injected until unit activity was suppressed as well.

17-20 cells were recorded; 16 phase and 1 tonic. Their pre-P0 mean discharge rate during baseline was 11.67 ± 3.13 Hz, while during large amplitude, irregular activity (LAA) it was 4.46 ± 2.76 Hz. At 1 min post-P0 the discharge rate was 5.345 ± 0.43 Hz. By the conclusion of the experiments, the mean rate had risen to 10.12 ± 3.352 Hz during 5.6 Hz, and 4.152 ± 2.632 Hz during LAA. 2.0 cells were recorded. Their pre-P0 mean discharge rate during 5.6 Hz was 6.284 ± 1.95 Hz. At 1 min post-P0 their mean discharge rate was 5.352 ± 2.397 Hz.

These data suggest that suppression of the MS/vDB removes excitatory inputs from on-cells (presumably cholinergic) in nature, and removes inhibitory inputs from off cells (presumably GABAergic). Research is now underway to examine the role of GABA in hippocampal field activity and cell activities.
448.11

THYROTROPIN-RELEASING HORMONE (TRH) IS RELEASED FROM HIPPOCAMAL SLICES AFTER ELECTROCONVULSIVE SHOCK (ECS). S.H. ENDLICH, S. DiBElfio, N.Y. Dept. of Anatomy, Psychiatry and Program in Medical Neurobiology, Indiana University Medical Centers, Indianapolis, IN 46202.

TRH levels (Ann. N.Y. Acad. Sci. 553:286-89) and TRH mRNA (this meeting) were elevated in rat hippocampus after ECS. We used an in vitro K+ stimulation preparation to examine whether increases in tissue TRH coincide with enhanced release. Sham ECS and ECS (3x) rats were killed either 12 or 24 h after the last treatment. Sliced hippocampi were perfused with oxygenated Kreb's solution modified as follows: (1) high K+ + Ca2+ (HKE); and (2) high K+ + EGTA (HKE).

Fractions were collected during a 30 min stimulation with K+ followed by HKE and analyzed for TRH via RIA. No significant release occurred in either group during HKE stimulation. TRH was increased in the HKE fraction of ECS animals compared to sham after both 12h (1.5+/-0.06 vs. 0.66+/-0.095 pg/mg.min. p<0.005) and 24h (2.11+/-0.32 vs. 0.74+/-0.16 pg/mg.min. p<0.02). HKE fractions contained more TRH than those obtained 12 h after ECS (p<0.025). This is the first report of TRH release from an extrahypothalamic site. Moreover, these results confirm that elevated TRH seen after ECS represents TRH available for release and thus may have a significant effect on receptor modulation. Supported by NS-25656 & VA Research.

448.13

DEPTH PROFILES OF AN ATROPINE-RESISTANT COMPONENT OF THE HIPPOCAMAL THETA RHYTHM IN URETHANE ANESTHETIZED RATS. Mark Stewart and Steven E. Fox, Dept. Physiol, SUNY Health Science Center, Brooklyn, NY 11203.

The hippocampal theta rhythm, first characterized by its distinct behavioral correlates, has been separated into atropine-sensitive and atropine-resistant components. The atropine-sensitive component of the urethane-induced theta rhythm is well known. Recently, an atropine-resistant component of this theta rhythm was described (Stewart and Fox, Brain Res. 500:55, 1989). We constructed depth profiles of the atropine-resistant component of the theta rhythm in urethane-anesthetized rats by averaging the hippocampal EEG recorded from different depths, triggered by the rhythmic activity of an atropine-resistant medial septal cell (Stewart and Fox, J. Neurophysiol. 61:1, 1989). Amplitude profiles showed peaks in the basal and mid-apical dendrites of CA1, near the granule cell layer. Amplitude minima were located at the pyramidal cell layer and in the apical dendrites of the dentate granule cells. Phase shifts of 90° occurred at the two amplitude minima so that the signals in the basal dendrites of CA1 and the dentate granule cell layer were phase-reversed. The largest amplitude peak occurred between the two 90° phase shifts suggesting that two different components are responsible for the profile. GABAergic septohippocampal afferents probably generate the amplitude peaks near the pyramidal and granule cell layers, but the origin of the peak in stratum radiatum is unknown.

(Supported in part by NIH grants NS17095 and NS07117.)

449.1

EXPERIENCE-DEPENDENT CHANGES OF SEXUAL PREFERENCES AND SPINE DENSITY IN A FOREBRAIN AREA OF THE ZEBRA FINCH. N.-J. Blischak and A. Rollenhagen, Dept. of Ethology, Univ. of Bielefeld, P.O. Box 9440, 4800 Bielefeld 1, F.R.G.

If a male zebra finch after 60 days of isolation courts a female the first time in its life, the birds under certain circumstances change their initial sexual preference. In this study we prove this change of preference and show that by such exposure to a female substantial changes of sexual behavior occur in one of four areas of the forebrain, which have been shown previously to be activated during this first courtship encounter.

The zebra finch readily zebra finch males which were after 60 days of isolation first exposed to a zebra finch female for 7 days followed by 7 days of exposure to Bengal finches, changed their preference to zebra finches, whereas males receiving the reverse exposure stabilized their initial Bengal finch preference.

The first 7 day exposure to a female after 100 days of isolation resulted in an increase in spine density in one subpopulation of ARC-neurons compared to controls.

We suspect that the features which are learned as releasers for courtship behavior in the course of the sensitive period have to be verified and consolidated in the first courtship encounter. The increase in spine density of ARC may be a reflection of this consolidation process, although it may also be a consequence of social experience per se. Supp. by Deutsche Forschungsgemeinsch.

449.2

MIDBRAIN AND TELENCEPHALIC CONTRIBUTIONS TO VOCAL CONTROL IN ZEBRA FINCHES D.S. Vicario and H.B. Sipman, The Rockefeller University, New York, NY 10021

Electrical stimulation has been reported to elicit vocalizations consistently from the avian midbrain, but rarely from the telencephalon. In songbirds, the vocal pathway includes telencephalic nuclei HVC and RA, the midbrain intercollicular complex (ICo), and the hypoglossal subregion (RPC) of the medulla projecting to the syrinx, or vocal organ. We have found that long microstimulation trains (1-4s) elicit vocalizations from HVC, RA, and ICo. Within ICo, the dorso-medial nucleus (DM) has the lowest threshold (<100µA). DM stimulation elicits loud, repeated vocalizations at short latency that resemble calls. In the caudal telencephalon, similar low thresholds for stimulation are obtained from HVC and RA. Vocalizations are softer, with longer latencies. They are acoustically complex, and can resemble syllables from the bird's own song in duration and/or acoustic features. Vocalizations elicited from RA lose their complex features if the syringleal nerve is cut. Since RA projects to both DM and nXHs, the stimulating vocalizations may depend on the RA to DM projection. These results suggest that some characteristic features of song are determined in the path from HVC to the syrinx, and can be evoked by a simple stimulus. We have also used stimulation to target lesions of DM in order to explore its contribution to normal vocalization in male and female zebra finches. We have previously shown that lesions of either HVC or RA abolish song and strip the male long call of its learned features, but do not affect the female long call. In contrast, bilateral lesions of either HVC or RA do not abolish song but primarily affect its temporal features: the motif duration is longer and sequence anomalies may increase, but only minor acoustic changes were seen. Vocalizations elicited from the midbrain pathway, in females with DM lesions, long call duration is dramatically affected. This suggests that vocal production in females is primarily due to the midbrain pathway, whereas in males, midbrain and telencephalic contributions interact.
**MASCULINIZATION OF ZEBRA FINCH VOCAL NUCLEI DEPENDS ON TIMING OF HORMONE TREATMENT.**


Telecephalic nuclei HVC and its two targets, RA and X, make critical contributions to learned vocalizations produced by male zebra finches. Female HVC does not produce these learned vocalizations; HVC, RA, and X are smaller in volume; HVC and RA are not connected. We have shown that castrating (E) balance is sufficient to masculinize the vocal behavior of females. We now present data showing that the timing of hormone treatment critically determines the spectrum of neuroanatomical masculinization.

Seven females received E pellets at birth. All produced male-like vocalizations. In 5 (30/100g, 25/50g), the volumes of RA and X were dramatically larger than in normal females, confirming earlier reports. HVC projections in these females included a greater number of neuroanatomical markers (Hна-1). The other 2 (100g) received testosterone (T) as adults and, in both, the frequency of singing increased; in 1, T treatment stabilized the temporal pattern, whereas the other female, T, had no further effect on the volume of RA or X.

Six females were treated with E at birth, but the pellets were removed at 15 days. In 4 of these, RA was small, but HVC projected to RA in only 2. In contrast, X was large in volume and was innervated by HVC. None of these females produced male-like vocalizations. The 2 remaining females received T as adults and then produced song-like vocalizations of poor quality. RA was small but innervated by HVC, X was large and innervated.

These data suggest 1) that the innovator and volume of X can be masculinized independently from that of RA, and 2) that RA innervation and RA size are not associated. We are now exploring the possible role of endogenous T in both behavioral and anatomical masculinization by treating females with Flutamide as well as E. (MH-40900; GMG-7739)

**ROLE OF LMAN IN POST-CRITICAL PERIOD SONG LEARNING IN ZEBRA FINCHES.**


Male zebra finches learn their songs during post-hatching days 30 and 80, but this "critical period" for song learning can be extended by isolating juveniles from singing adults (Eales 1985, 1987). Removal of the lateral magnocellular nucleus of the anterior neostriatum (LMAN) of normally reared zebra finches disrupts song development and affects production of adult males (Bottjer et al., 1984). To determine whether the effects of LMAN removal depend on age or on completeness of learning, we evaluated the role of LMAN in isolates after the normal end of the critical period.

Male zebra finches were visually isolated from conspecifics from day 28 to 80, when their songs were recorded. In group I an adult conspecific male tutor was then added to each cage; in group II each isolate was given HVC lesions and then an adult male was added. All birds were kept with their tutors for 4-5 months, and songs recorded periodically.

After 4-5 months of tutoring, intact isolates had learned an average of 4 elements of their tutors' songs; in contrast, lesioned isolates retained their songs but showed no evidence of new learning. These results suggest that in adult zebra finches that can still modify their songs, LMAN is necessary for song learning but not for maintenance of song already developed.

**EFFECTS OF EARLY AUDITORY DEPRIVATION ON THE DEVELOPMENT OF AVIAN VOCAL CONTROL NUCLEI.**


Altering sensory experience can change the timing of neural events associated with perinatal time periods. Male zebra finches, the sensitive period for vocal learning correlates with large changes in the size and number of song-related neurons. Between 20 and 65 days of age, song-related neurons are added to the hyperstriatum ventralis pars caudalis (HVC) and Area X, and the robust nucleus of the archistriatum (RA) increases in volume. In contrast, the lateral magnocellular nucleus of the anterior neostriatum (IMAN) loses nearly 50% of its volume during this critical period. To determine how auditory experience during the sensitive period affects these changes, male zebra finches were deafened or sham operated at 10 days of age and sacrificed 65 days later. Nuclear volume and neuron number were measured for HVC, RA, IMAN and area X.

Early deafening did not alter the development of neuron number in HVC, Area X or RA, but did attenuate neuron loss from IMAN. At 65 days, nuclear volume and neuron number in HVC, Area X, and RA did not differ between intact and deafened birds. In contrast, while both groups lost IMAN neurons between day 25 and 65, at the latter age IMAN neuron number was significantly less in intact than in deafened birds. These data suggest that auditory experience during the sensitive period regulates neuron death in IMAN. To confirm these findings and identify the neurons spared by auditory deprivation, we are extending this study using retrograde and anterograde tracing techniques.

**ACQUISITION OF A CONSPECIFIC SONG DISCRIMINATION IS FACILITATED BY TESTOSTERONE IMPLANTS IN CASTRATED ZEBRA FINCHES.**


Adult male zebra finches learned to discriminate between conspecific songs in fewer trials when trained in the summer than in the winter. We speculated that the increased critical period in winter results from differences in testosterone (T) levels. To test this hypothesis, castrated zebra finches were implanted with either an empty, an empty-scalpel, or empty-scalpel + testosterone (T) pellets. We then trained the birds to discriminate between two canary song segments. Hopping to the food dispenser after hearing one song segment was rewarded with access to seeds; hopping to the dispenser after hearing the other song segment resulted in a time out. All birds required approximately the same number of trials to acquire the discrimination. Each T-implanted bird was then paired with an empty-scalpel implanted bird. Each pair was then trained to discriminate between their own two songs. T-implanted birds acquired the discrimination faster than their empty-scalpel paired controls. The results suggest that testosterone facilitates acquiring a conspecific, but not heterospecific song discrimination.
449.9
BLOCKING STEROID HORMONES DURING VOCAL LEARNING EXTENDS SENSITIVITY TO DEAFENING IN ZEBRA FINCHES. S.W. Bottjer & E. Foster. Dept. Biol., USC, Los Angeles, CA 90089

Depauperization of male song behavior in zebra finches, indicating that they require auditory feedback of vocalizations in order to develop normal song. However, deafening exerts little or no effect on adult song (max. = 16, 1990 days) that are producing stereotyped song. We have previously reported that blocking steroid hormones prevents normal development of learned vocal behavior by delaying exposure to neuronal input. However, this delay is insufficient in the normal period for vocal learning permits delayed development of normal song production. This pattern of results raises the possibility that blocking steroid hormones could extend the period during which zebra finches can learn to produce specific song patterns, and that high levels of testosterone and/or the development of stereotyped motor patterns of song act to close the period for learning.

To test this idea, males were castrated at 20 days of age and received continuous exposure to an anti-androgen (flutamide) and/or an anti-estrogen (tamoxifen) until g. 185 days of age. Birds were then deafened via removal of the cochlea, and song behavior was recorded pre-operatively and for a period of one to two months following surgery. In all cases deafening produced significant disruption of the song patterns even though birds were well beyond the normal period of susceptibility to deafening. Thus, pre-disruption of normal song behavior by blocking access to sex steroids seems to extend the period of reliance on auditory feedback.

449.11
NEURONAL RESPONSES TO SONGS AND ARTIFICIAL STIMULI IN OVODALIS AND HVC: A CONNECTIONIST MODELLING APPROACH. S. C. Banker* & D. Margoliash. Rand Corp., Santa Monica, CA and Dept. Anatomy, Univ. of Chicago, Chicago, IL.

A quantitative relationship between centrifugal neuronal responses to simple artificial and complex natural stimuli has rarely been achieved. We conducted recordings in urethane-anesthetized zebra finches and backpropagation experiments to investigate this issue. Auditory neurons in the song system nucleus HVC responded well to song, systematically preferring the bird’s own song. Some but not all HVC neurons also responded to various artificial stimuli tested. In contrast, ovoidalis neurons responded to tones as well as song, and exhibited other classical properties.

For modelling, modified backprop architecture we are experimenting with utilize trainable feature detectors with limited temporal windows whose response across the duration of the stimulus is integrated by output units whose temporal response properties are also constrained. A two phase training technique where initial weights for feature detectors are set via pre-training using short duration artificial stimuli has thus far been the most effective. Current interest in this work consistently shows excitability to the stimuli which evoke responses in the neurons, but have some susceptibility to false alarms. These limitations may be resolved with use of different, higher order, or fully recurrent architectures.

These results can provide a quantitative framework for identifying nonlinear neuronal properties, transformations in the auditory pathway, and can provide a rationale when using a limited stimulus repertoire. Supported by grants from ONR and NIH. DM is a Starr Scholar.

449.10
LESIONING AXONAL CONNECTIONS OF A THALAMIC NUCLEUS DISRUPTS SONG DEVELOPMENT IN ZEBRA FINCHES. K.A. Halama & S.W. Bottjer. Dept. Biol., USC, Los Angeles, CA 90089

Previous investigations suggest that discrete components of the neural system in male zebra finches play a critical role in the development of song, but not in the production of the stable adult song. Area X is projected to IMAN by an antisynaptic, via the thalamic nucleus DLM. In order to investigate the role of DLM in song learning, we made electrolytic lesions of a forebrain region containing both the anterior and DLM from Area X and examined the effects from DLM in IMAN in young males between 40 and 70 days of age. In all instances when these two sets of fibers were damaged, vocal production was significantly disrupted.

Surprisingly, unlike lesions of IMAN, the disruptive effect of this type of lesion on the final adult song did not fall off as the bird developed a more usual song pattern. Analysis of the adult song in birds over 100 days of age revealed that lesions made as late as 70 days produced vocal deficits comparable to lesions made earlier in development. In contrast, disruption of these fibers in adult birds left song production unaltered. The X-DLM-IMAN circuit appears to be developmentally regulated at lesions made only early in ontogeny and modifies song production. However, the afferents to IMAN are effective later in vocal development than are lesions of IMAN itself. Thus, not all portions of this circuit "orchestrate" song development within the same time frame.

449.12
PERCEPTION OF BIRDSONG BY FEMALE ZEBRA FINCHES & CANARIES. S.J. Clark and F. Nottebohm. The Rockefeller University, Field Research Center, Millbrook, NY 12545.

Birdsong serves two roles - the spacing of males and the wooing of females. If female mate choice is influenced by song, then female preferences will shape the predispositions of males to learn some songs over others. However, males and females need not perceive song in the same way. Here we report our first attempt at defining the song parameters perceived and preferred by female zebra finches and canaries.

Using playback of songs to estradiol implanted-female canaries and zebra finches, we were able to elicitcopulation solicitation displays in the laboratory and used these stimuli to test if females prefer conspecific song but not heterospecific song. The songs of conspecific males raised in isolation were much less effective than the songs of normally raised males in evoking solicitation displays. Using a computer to manipulate parameters and modify songs revealed that female zebra finches appear to attend to a hierarchy of cues with predictable structural morphology being more important than constant sequence of syllables.

Female canaries also discriminate between conspecific and heterospecific songs. Female canary song does elicit displays but is not as effective as male song. We are now searching for features of conspecific song that can be heightened so as to elicit super-normal responses in females. It is our hope that finding such features will tell us a good deal about the mechanisms involved.

450.1

Marijuana is the most widely abused drug in the United States. Yet there is little known about its actions on the A9 mesocorticolimbic dopamine systems which are considered of fundamental importance for the positive reinforcing effects of many drugs of abuse. These studies were set out to determine the effects of delta-thc, the major psychoactive constituent of marijuana, on A9 neuronal activity.

Under chloral hydrate anesthesia, extracellular recordings were made from single A9 neurons during i.v. injections of delta-THC, cannabinol (a nonpsychoactive cannabinoid) or vehicle. Evaluation of the cumulative dose-response curve revealed that delta-thc caused a dose-dependent increase in firing rate reaching a maximum 19% change at 4 mg/kg. However, larger doses led to some attenuation of the increase, possibly due to the depressant effects of the vehicle. Identical doses of cannabinol produced virtually no effect (max. = 3%), while equivalent volumes of vehicle (4 ml/kg) resulted in a progressive decrease in rate by 13%. Further experiments indicate that delta-thc stimulated responses which were not reduced by 5-HT2 antagonists. However, the apomorphine-induced inhibitions of A9 firing do not appear to be different following vehicle or delta-thc treatment. Since the vehicle in which delta-thc is solubilized was antagonistic, delta-thc may actually be a more potent activator of mesocorticolimbic dopamine neurons than demonstrated in this preparation.

Thus delta-thc has a significant effect on A9 activity, like other drugs of abuse. It remains to be determined whether this action might underlie some of marijuana's abuse liability and psychotropist properties.

450.2

We have previously shown that delta-tetrahydrocannabinol (delta-THC), the psychoactive ingredient of marijuana, augments brain-stimulation reward (Reichardt et al., Pharmacol. Ther. 30:109-16, 1986). These studies are now designed to test the hypothesis that a delta-THC effect on DA efflux in caudate-putamen (Ng Cheong Ton et al., Brain Res. 451:25-39, 1988) and in nucleus accumbens and medial prefrontal cortex (Chen et al., Proc. Natl. Acad. Sci. 86:7240-4, 1989). Further, the delta-THC dynamics of delta-THC’s effect on DA efflux suggested an action analogous to that of a DA reuptake blocker (Ng Cheong Ton et al., Brain Res. 451:59, 1988) and in nucleus accumbens and medial prefrontal cortex (Chen et al., Proc. Natl. Acad. Sci. 86:7240-4, 1989). Further, the delta-THC dynamics of delta-THC’s effect on DA efflux suggested an action analogous to that of a DA reuptake blocker (Ng Cheong Ton et al., Brain Res. 451:59, 1988) and in nucleus accumbens and medial prefrontal cortex (Chen et al., Proc. Natl. Acad. Sci. 86:7240-4, 1989). Further, the delta-THC dynamics of delta-THC’s effect on DA efflux suggested an action analogous to that of a DA reuptake blocker (Ng Cheong Ton et al., Brain Res. 451:59, 1988) and in nucleus accumbens and medial prefrontal cortex (Chen et al., Proc. Natl. Acad. Sci. 86:7240-4, 1989). Further, the delta-THC dynamics of delta-THC’s effect on DA efflux suggested an action analogous to that of a DA reuptake blocker (Ng Cheong Ton et al., Brain Res. 451:59, 1988) and in nucleus accumbens and medial prefrontal cortex (Chen et al., Proc. Natl. Acad. Sci. 86:7240-4, 1989).

The present experiments were undertaken to further explore that possibility, using intracerebral microdialysis in awake freely-moving rats. We now report that haloperidol (0.1 mg.kg./p.) pretreatment (1 hr prior to delta-THC) has a synergistic effect on delta-THC’s enhancement of presynaptic DA efflux in nucleus accumbens. Delta-THC (1.0 mg.kg., p.) pretreatment 1 hr before haloperidol also had a synergistic effect on presynaptic DA efflux. "Tetradeflon" perfused locally at 10^-6 M abolished the synergism between haloperidol and delta-THC. These results indicate that haloperidol- induced facilitation of DA release underlies the synergistic effect between DA receptor blockers and DA-reuptake inhibitors (Westenf et al., Eur. J. Pharmacol. 153:125, 1987), the present data add further evidence that delta-THC may act as a DA reuptake blocker in brain reward circuitry.
50.3 DELTA-9-TETRAHYDROCANNABINOL DOES NOT AFFECT EFFLUX OF MESOTELENCEPHALIC Dopamine AS MEASURED BY IN VIVO MICRODIALYSIS IN FREELY MOVING RATS. S. D. 00964, E. Castañeda, D. B. Bowman. Neurosciences Institute, University of Lethbridge, Lethbridge, Alberta T1K 3M4, Canada, and University of Texas, El Paso, Texas 79999. We used in vivo microdialysis to evaluate whether delta-9-tetrahydrocannabinol (THC) alone increases the efflux of dopamine (DA) from the Striatum or nucleus accumbens and whether THC enhances the increase of DA produced by either amphetamine or phendrehemine. Dialysate samples were collected (1) before, (2) after 1 mg/kg or 10 mg/kg THC (or vehicle), and (3) after 1.5 mg/kg d-amphetamine (DA). Locomotor responses and turning behavior were measured during these three conditions, an additional experiment 10 mg/kg THC (or vehicle) plus 0.3 mg/kg phendrehemine (2HC) was administered immediately after morning-state (baseline) measures. THC produced no change in extracellular concentrations of DA, DOPAC, and HVA, and in 5-HTAA, and had no effect in DA release produced by amphetamine and phendrehemine. There were no behavioral differences between groups, including during any treatments. Thus, increasing activity at mesotelencehalic DA synapses is an unlikely basis for the propensity to self-administer marijuana by humans.

50.4 QUANTITATIVE TOPOGRAPHIC EEG STUDIES OF CHRONIC THC ABUSE. F.A. Struve, J.J. Strounangis, G. Patrick* and I. Katz*. Neurophysiology Lab, LSU Sch. of Med., New Orleans, LA 70112. In a pilot study (Clim. EEG., 19:6-23, 1989) 10 chronic daily THC users with no THC access and 90 non-users were contrasted with 20 current THC non-users and 10 normal non-users using multiple quantitative EEG measures. THC users had significant elevations of Absolute and Relative Power and Coherence of alpha over frontal cortex ("HYPERFRONTALITY OF ALPHA") as well as other quantitative EEG signs. These findings have subsequently replicated with new samples of 17 THC users, 21 patient non-users and 12 normal non-users. Also using all 80 Se and all quantitative EEG variables as potential predictors, a discriminant function analysis yielded a 95% correct THC user vs non-user classification. In expanded studies Discriminant Scores correlated positively with Duration of THC use in years and Exposure (Duration x Daily Amount) but negatively with duration of Abstinence in years. Finally, the spectral profile (Absolute Power in 1 Hz intervals from 1 to 25 Hz) was found to be shifted to slower frequencies for THC users as compared to non-users.

50.5 DELTA-9-TETRAHYDROCANNABINOL EFFECTS ON TONE EVOKEPOTENTIALS RECORDED FROM RAT NECOCORTEX. Sam A. Deadwyler, Eric M. Bladioc* Jan Ma* and Robert E. Hampson. Department of Pharmacology and Physiology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina 27103. Previous studies from this laboratory have shown that administration of delta-9-tetrahydrocannabinol (THC) impairs behavioral detection of a single tone in rats performing a signal detection task (Heyser et al., SN Abstr., 14:1030, 1980). The same THC dosages (0.5-2.0 mg/kg) did not alter neural activity associated with auditory system detection of the tone (Campbell et al., JPET, 239:941-945, 1986) but reduced tone-related activity associated with behavioral responding to the tone. We recorded cortical EPs from skull electrodes in the rat to determine whether the effect of THC on processing of behaviorally-relevant sensory information is dependent on the hippocampus. Cortical EPs evoked by brief (4 msec) tone "pips" revealed several short-latency auditory system-related potentials (+10 usec). Large negativities at 12 msec (N12), 25 msec (N25), and 40 msec (N40) were also evoked by the tone.

50.6 DELTA-9-TETRAHYDROCANNABINOL INHIBITION OF THE NEUROENDOCRINE RESPONSE OF ADULT MALE RATS TO SEXUALLY RECEPTIVE FEMALE CONSCALLIPSE. G. Cherp, L.L. Murphy, B.W. Steger and A. Batker*. Department of Pharmacology, Southern Illinois University, School of Medicine, Carbondale, IL 62901. The effects of delta-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, on LH and prolactin (PRL) levels and hypothalamic norepinephrine (NE) turnover in adult male rats exposed to sexually receptive female conspecifics were investigated. Adult male Sprague-Dawley rats were given po. either sesame oil vehicle (control) or THC (5.0 mg/kg, b.w.) and 40 min post-treatment were either placed in an empty cage or a cage containing a sexually receptive female. Males were sacrificed 20 min after exposure (60 min after THC or vehicle treatment) for measurement of LH and PRL levels.

50.7 NALOXONE REVERSSES BUT DOES NOT PREVENT DELTA-9-TETRAHYDROCANNABINOL-INDUCED INHIBITION OF PULSATILE LH SECRETION IN OVARIECTOMIZED RATS. P. Keller*, M. Kohli* and L.L. Murphy, Department of Physiology, Southern Illinois University, School of Medicine, Carbondale, IL 62901. Although the ability of delta-9-tetrahydrocannabinol (THC), the chief constituent of marijuana, to suppress LH secretion in laboratory animals has been clearly demonstrated, the mechanism of this THC action has yet to be elucidated. In the present study, the ability of naltaxone (NAL), an opiate receptor blocker, to reverse or to modify the effects of THC on pulsatile LH secretion was examined in ovariectomized Sprague-Dawley rats. Approximately 4 weeks post-ovariectomy, blood samples were drawn, via indwelling intra-aquatorial canules, every 5 min for 60 min pre- and 120 min post-vehicle (control) or THC (0.5 mg/kg, b.w., i.v) treatment at time 0 for LH determinations. Rats were administered NAL (2 mg/kg, i.v. or i.v) saline at 20 min post-treatment, time 0, and/or at 30 min post-treatment. The results indicated that THC treatment alone significantly reduced plasma LH levels by 20 min post-treatment to 50-75% of control values (p<0.05). Treatment with NAL did not prevent THC-induced suppression of LH release. However, NAL administration at 20 min post-THC treatment reversed the inhibitory effect of THC on LH (p<0.05). Moreover, when NAL was administered prior to THC, concomitant with THC administration, and 20 min post-THC treatment, the magnitude of LH suppression by THC and the duration of suppression were significantly attenuated. These data suggest that endogenous opiate peptides play a role in the ability of THC to inhibit pulsatile LH secretion, however, the possibility that other CNS mechanisms (e.g., norepinephrine) are involved is strongly implicated. (Supported by DA 5042)

50.8 EFFECTS OF δ9-TETRAHYDROCANNABINOL ON REGIONAL CEREBRAL BLOOD FLOW IN THE RAT. A.S. Bloom, S.A. Fuller*, E.A. Stein. Departments of Pharmacology and Psychiatry, Medical College of Wisconsin, Milwaukee, WI 53226. δ9-Tetrahydrocannabinol (THC), the principal psychoactive ingredient in the marijuana plant, produces a wide range of behavioral and physiological effects. Cannabinoid binding sites have been found to be widely distributed throughout the brain. In order to better determine functional sites of action for the cannabinoids, we have now examined the effects of THC on regional cerebral blood flow (rCBF) in the rat. Conscious rats were injected IV or 16 mg/kg THC or its Emulphor ethanol vehicle 30 min prior to sacrifice. [14C]iodoantipyrine was used to determine rCBF according to the method of Sakurada et al. (J. Cereb. Blood Flow Metab., 4:99-53, 1984). Two regions were measured. Two patterns of change were observed. A significant dose related decrease in rCBF was seen in the hippocampus (up to 30%) and its major input and output regions, the lateral and ventromedial hypothalamic nuclei, and the dentate. In contrast, a dose-related biphasic increase (4 mg/kg>16 mg/kg) in rCBF was seen in such areas as the anterior, lateral, and ventromedial hypothalamic nuclei involving an autonomic regulation. Other areas such as the medial septum and claustrum were unaffected. It is significant that the areas where rCBF is altered are involved in the major behavioral and physiological effects of THC such as impairment of short-term memory and increased autonomic activity. The results of this study indicate that alterations in rCBF may be a sensitive technique for the localisation of cannabinoid sites of action within the brain. (Supported in part by NIDA grants DA03725 and DA05012)
450.9


Theoretical analysis of the neuropharmacological basis of negative reinforcement has been hampered by difficulties in generating baseline performances comparable to those used with positive reinforcement. The timeout from avoidance procedure permits the study of negatively-reinforced behavior under schedules ordinarily used only with positive reinforcement. Rats were trained on concurrent schedules where responses on one lever produced shock (aversion) and responses on another lever produced signaled intervals of timeout from avoidance during which the avoidance schedule was suspended. Timeout was programmed on a variable-interval 45-s schedule, and generated patterns of behavior similar to those produced by comparable schedules of positive reinforcement. Morphine depressed responding on the timeout lever at doses that increased or did not affect avoidance. Amphetamine selectively increased responding on the timeout lever, but chlorpromazine and buspirone non-selectively decreased responding on both levers. The finding that the effects of some drugs (morphine and amphetamine) depend on the type of negative reinforcer maintaining behavior has implications for theories regarding the neurochemical substrates of negative reinforcement.

450.11

SYSTEMIC NICOTINE INDUCES NEURONAL FOS IMMUNOSTAINING IN DISCRETE BRAIN REGIONS. S.M. Savar*, Dept. of Neurology, Univ. of California, and VA Medical Center, San Francisco, CA 94113.

Nicotine, 2 mg/kg s.c., was administered to Long Evans rats which were sacrificed 1 hr later and processed for Fos immunocytochemistry using a polyclonal antibody that recognizes authentic and fos-related antigens. Brain regions with high levels of Fos immunostaining following nicotine administration include the hypothalamic (median eminence, arcuate nucleus, paraventricular nucleus, supraoptic nucleus), hippocampus, cortex, thalamus, and pontine nuclei. Fos expression was widespread across a variety of neuronal populations. These observations demonstrate that nicotine is capable of stimulating Fos synthesis in vivo, although the mechanism is not necessarily direct; (2) some, but not all, neurons with nicotinic receptors synthesize Fos in response to nicotine; and (3) Fos immunocytochemistry may be a useful tool to investigate the effects of nicotine on the central nervous system.

450.12

PHENCYCLIDINE-INDUCED ALTERATIONS OF EXTRAPYRAMIDAL AND LIMBIC NEUROPEPTIDE Y SYSTEMS. L.P. Midgley, L.G. Bush, J.W. Gsbb and G.R. Hanson, Dept. of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.

Phencyclidine-HCl (PCP) causes many effects including behavioral changes which resemble the negative and positive signs seen in schizophrenia. PCP is known to interact with a number of transmitter systems and has recently been identified as an NMDA antagonist. We postulated that administration of PCP significantly reduces striatal neuropeptide Y (NPY) levels in rats: some of these NPY changes also occurred after treatment with the non-competitive NMDA antagonist MK-801. In the present study, we observed that PCP administration also caused significant declines in NPY levels scattered throughout the neocortex and thalamus, but not in the substantia nigra. In order to elucidate the nature of these responses, selective D-1 (SCH 3390), D-2 (sulfuride), 5-HT-1 (CGS-15943) antagonists and a drug which depletes serotonin (PCA) were administered alone and in combination with PCP. Only SCH 3390 attenuated the PCP-induced change in NPY levels in all structures examined. These results suggest that D-1, but not D-2, 5HT or sigma receptors play a role in PCP-induced changes of extrapyramidal and limbic NPY systems. (Supported by grants DA 00869 and DA 04222).

450.10


We have previously shown that changing environmental cues associated with drug delivery disrupts tolerance to the analgesic and anorectic effects of nicotine in rats. Here we asked whether the effectiveness of nicotine in activating the hypothalamic-pituitary-adrenocortical system, as measured by blood corticosterone levels, can also be brought under environmental control. Secondly, we were interested in further characterizing the nature of this environmental control. Male rats developed tolerance to the anorectic and corticosterone elevating effects of daily injections of 0.33 mg/Kg of nicotine bitartrate. When cues uniquely associated with drug delivery were then changed, both behavioral and corticosterone tolerance were disrupted. When there were no unique cues associated with drug delivery per se, changing the environment had no effect on tolerance to either of nicotine's actions. Thus conditioning principles apply to tolerance of the neuroendocrine, as well as behavioral effects of nicotine.

Discontinuation of the triazolobenzodiazepine alprazolam (ALP) can produce a clinical "withdrawal" syndrome. An analogous syndrome of behavioral hyperactivity and GABA_A receptor upregulation has been reported in mice (Lopez et al., Neuropharmacol, 29:237, 1990). Some clinical evidence indicates that carbamazepine (CBP) may be useful in limiting the symptoms associated with alprazolam discontinuation.

To evaluate the effects of CBP on ALP discontinuation in a mouse model, we assessed the effects of an anticonvulsant dose of CBP (50 mg/kg/d) administered on open-field activity, benzodiazepine binding in vivo and in vitro, TBPS binding, and GABA-dependent chloride uptake in cortex. ALP (2 mg/kg/d) or vehicle was administered for 1 week before being given 1 wk treatment with vehicle or by intraperitoneal injection, benzodiazepine or TBPS binding, or GABA-dependent chloride uptake. As previously reported, ALP followed by vehicle was associated with increases in motor activity, benzodiazepine binding, and chloride uptake 24 h and 4 days after ALP discontinuation. Similar findings were observed in mice treated with CBP after alprazolam. Thus, CBP at an anticonvulsant dose does not affect behavioral or neurochemical effects of alprazolam discontinuation.

451.4 EFFECTS OF EARLY EXPERIENCE ON DRUG CHOICE IN A NOVEL ANIMAL MODEL OF MULTIPLE SUBSTANCE ABUSE. N.Zimmerberg and M.S.Brett*. Psychology Dept., Williams College, Williamstown, MA 01267.

Individuals vary in rates of initiation and maintenance of drug self-administration behavior. One source of these individual differences may lie in differential early experience. Long-Evans rats were weaned at 25 days of age into one of three environmental conditions: social isolation (SI), sibling double-housed (DH), or enriched environment (EE). At 85 days of age, subjects were singly housed and given choice between drinking a stimulant (d-amphetamine, 0.025 mg/ml), a depressant (barbitual, 1 mg/ml) or water for two home cage sessions. The choice was affected by both housing and sex. SI males consumed significantly less depressant than any other group. These results suggest that males and females respond differently to the stress of social isolation, and appear to contradict the "tension-reduction" hypothesis used to explain depressant drug intake, at least for males.

451.5 TOLERANCE TO PHENOBARBITAL AND CROSS-TOLERANCE TO MUSCIMOL GIVEN I.V. TO RATS. J.A. Richter, S.L. Gilbert* and B. Glick*. Dept. Pharmacology and Psychiatry, Indiana Univ. School of Medicine, Indianapolis, IN 46202.

We have found previously that many barbiturates (Rhe) effects including the loss of righting reflex (RIR) and hypothermia can also be blocked by iv administration of muscimol. In order to test the hypothesis that Rhe induces these effects by a GABAergic mechanism, we tested if cross-tolerance occurs between these two drugs. Studies were done in male Wistar rats with indwelling guide canulae directed to the lateral ventricle. The methods were previously described by Myslak and Iresonoff (1976) who found tolerance to the RIR induced by MePh after 4 days of 4x/day iv injections. We found that 1250 mg NaMeph 4x/day for 4 days induced tolerance to the RIR but not to the hypothermia. Similar chronic administration of 1600 mg NaMeph induced tolerance to the hypothermia and RIR. When these rats were tested on the 5th day with 1 mg muscimol, the hypothermic effect of muscimol was reduced significantly, but the RIR was not. These results support a GABAergic mechanism for barbiturate hypothermia. Further experiments will test whether long-term treatment will induce cross-tolerance to the other effects of muscimol, and if chronic muscimol induces cross-tolerance to Rhe effects. (Supported by NIMH RO1 DO0796).


This investigation explored the importance of chronic dose on behavioral tolerance and behavioral withdrawal with midazolam (Mz). Forty-eight male rats were trained to respond on a fixed ratio (FR) 30 schedule of reinforcement during daily 20 min. sessions. An initial dose effect curve was determined (0.3–10.0 mg/kg) and de-rate was determined following 30 days on chronic FR. During the chronic exposure period half the rats (N=24) were exposed to drug 15 min. preexposure (PRE) and half were exposed 30 min. postexposure (POST) with 6 rats from each PRE and POST group exposed to 0.1, 3.0 or 10 mg/kg during the chronic period. PB performance was observed following termination of drug exposure. Behavioral withdrawal also did develop to Mz with the degree of behavioral activity decreasing. The behavioral withdrawal was also determined by time of chronic drug exposure and Mz dose delivered. These findings support the hypothesis that behavioral factors are important in the development of tolerance and withdrawal and that behavioral factors are optimal when chronic drug delivery does not produce complete debilitation. Support DA05253.
451.7 WITHDRAWAL AND CROSS TOLERANCE IN RATS MADE DEPENDENT ON CHLORIDIAZEPoxide. L. L. Pugh†, S. Abdel-Malek*, and M. W. Emmett-Giles†, Department of Pharmacology, TCOM, Fort Worth, Texas, 76179-2960.

Rats were trained to detect the anxiogenic drug pentylenetetrazole (PTZ; 20 mg/kg) using a two-lever food-reward choice task. When tested with chlordiazepoxide (CDP; 20 mg/kg) in combination with the benzodiazepine antagonist, flumazenil (40 mg/kg), they selected the saline lever. Subsequently, they were treated with CDP (20 mg/kg/4-h) for 7 days, and then they were retested with CDP/flumazenil at various times after stopping chronic administration of CDP. In contrast to the results obtained prior to chronic CDP, this combination of drugs now resulted in predominating the saline lever selection at 8 and 24 h after CDP/flumazenil administration. However, in acute dose-effect tests, PTZ substituted for CDP, and flumazenil, CDP and diazepam substituted for midazolam in a dose-related manner. In addition, low doses of drug resulted exclusively in either responding on the saline lever or responding on the lever that was appropriate for that drug (e.g., PTZ-lever following PTZ). These subjects were then given CDP (20 mg/kg/4-h) for 7 days and subsequently tested for their detection of midazolam. The dose-effect curve shifted approximately two-fold to the right at 6 h after the last chronic injection of CDP. Thus, these results support the hypothesis that chronic CDP tolerance depends on the ability to detect a benzodiazepine and saline. These data are consistent with having trained the animals to discriminate saline from each of the drugs tested.

Supported by grant DA RO1 3521.


To assess dependency liability, squirrel monkeys received Ro 16-6208 (RO), aza-ropamol (A) or vehicle po divided into 3 equal doses at 8 h intervals each day over 11 days. Benzodiazepine receptor (BZAR) antagonist challenge (25 mg/kg Ro 15-3505 iv) was given 5 h, 24 h, and 46 h after the 3rd and final administration and observation period for 2 h. Pronounced convulsions were observed in 2/4 and 4/4 of the monkeys receiving 1 or 3 mg/kg A daily, respectively. In contrast, only 0/4, 1/4, and 2/4 of the monkeys receiving 3, 10, or 30 mg/kg of RO daily exhibited any pronounced convulsions, respectively. Given the much greater potency of RO than A in diverse pharmacological tests and the relative low incidence of withdrawal symptoms after the former, RO clearly exhibits lowerdependence potential than the BZAR full agonist. A pattern of results was repeated in DBA/2J mice receiving 2 or 20 mg/kg A, 20, 60, or 200 mg/kg RO or vehicle divided into two equal daily oral doses for 17 days and challenged with Ro 15-3505 given 5 h after the final administration. Convulsions were precipitated in 60-70% of mice in groups treated with the two higher doses of A, whereas none were induced in any of the RO groups. To assess its reinforcing effect, RO was offered to 4 cynomolgus monkeys which had previously been trained to self-administer pentobarbital on a FR10 schedule during a daily 6-h session. In repeated substitution tests (consisting of at least 5 sessions), RO was shown 1 lack reinforcing effects at all doses tested (0.001-9.5 mg/mg per injection). Furthermore, these doses were also inactive in 5 monkeys tested in a self-administration initiation paradigm. (For comparison, it has been found that oral RO inhibited punished operant responding in squirrel monkeys with a MED of 0.02 mg/kg). Thus, RO was not found to be self-administered within a behaviorally active dose range.


Psychology Dept., Rutgers Univ., New Brunswick, N J 08903

We selectively bred rats differing in degree of negative contrast effect when shifted from 32% to 4% sucrose. Shift ratio (mean frequency on first frshd day/llck frequency on last prefrshd day) was used as the selection criterion. Reliable differences between the two lines (large contrast sibs) and small contrast sibs) developed over six filial generations. Selection in the direction of contrast larger than the general population was much more pronounced in line selection toward the small contrast, an outcome which reflects the distribution of individual differences in a large sample of unselected rats. The two lines differ also in operant behavior in sensitivity to the contrast- alleviating effects of midazolam -- with the drug reducing contrast in the LC rats but having no effect in the SC rats. The two lines also differ in open field activity (LC rats ambulate and rear more than SC rats), but they do not differ in defecation frequency.

The two lines may provide a vehicle for analyzing biological and biochemical correlates of sensitivity to absolute and relative reward effects. The lines may also differ in sensitivity to excitotoxic drugs or possibly, other drugs which interact with reward systems.

451.8 DISCRIMINATIVE STIMULUS PROPERTIES OF FLUMAZENIL DURING CHRONIC CHLORIDIAZEPoxide TREATMENT G.A. Rowan and M.W. Emmett-Giles†, Department of Pharmacology, TCOM, Ft. Worth, Texas 76179-2960.

Rats were trained using operant methods to discriminate the stimulus properties of flumazenil (2.5 mg/kg) while maintained on a liquid diet containing chloridiazepoxide (CDP) (100 mg/kg/daily). The animals were given CDP in divided doses: they were trained 6 hours after the benzodiazepine antagonist, flumazenil (40 mg/kg), they selected the saline lever. Subsequently, they were treated with CDP (20 mg/kg/4-h) for 7 days, and then they were retested with CDP/flumazenil administration at various times after stopping chronic administration of CDP. In contrast to the results obtained prior to chronic CDP, this combination of drugs now resulted in predominating the saline lever selection at 8 and 24 h after CDP/flumazenil administration. However, in acute dose-effect tests, PTZ substituted for CDP, and flumazenil, CDP and diazepam substituted for midazolam in a dose-related manner. In addition, low doses of drug resulted exclusively in either responding on the saline lever or responding on the lever that was appropriate for that drug (e.g., PTZ-lever following PTZ). These subjects were then given CDP (20 mg/kg/4-h) for 7 days and subsequently tested for their detection of midazolam. The dose-effect curve shifted approximately two-fold to the right at 6 h after the last chronic injection of CDP. Thus, these results support the hypothesis that chronic CDP tolerance depends on the ability to detect a benzodiazepine and saline. These data are consistent with having trained the animals to discriminate saline from each of the drugs tested.

Supported by grant DA RO1 3521.


Rats (N=6) were trained to discriminate the stimulus properties of the benzodiazepine (BZ) receptor inverse agonist DMC from saline in a conditioned taste aversion paradigm. On a drug trial, water-deprived rats were injected with DMC (4 mg/kg IP), allowed access to a 25% saccharin solution for 30 min, and then injected with LiCl (1.8 mg/kg IP). On nondrug trials, saline injections bracketed the drinking period. Conditioned controls (N=6) were treated with DMC but never received LiCl. Acquisition of the discrimination was detected as few as 3 pairings of DMC/LiCl. In addition, DMC produced a dose-dependent reversal of the preference for saccharin over water in a two-bottle choice test after DMC/LiCl pairings.

The inverse agonists beta-CCE (10-18 mg/kg) and FG-7142 (3-2.2 mg/kg) fully substituted for the effect of DMC, as measured by the reversal of saccharin preference. Partial substitution for DMC was shown by the BZ receptor antagonist GUR 216 (3.2-10 mg/kg) and the non-BZ convulant pentylentetrazol (31-20 mg/kg). The BZ agonists chloridiazepoxide (0.32-5.0 mg/kg), diazepam (3.2-10 mg/kg), and alprazolam (0-1.3 mg/kg) all failed to generalize significantly to the DMC stimulus.

451.12 EFFECT OF ACUTE PENTOBARBITAL ON EXCITATORY AMINO ACID EVOKED-ELEVATIONS OF CYCLIC GUANOSINE 3’5’ MONOPHOSPHOSPHATE IN CULTURED CEREBELLAR GRANULE CELLS. W. W. Morgan, J. L. Bermudez* and S. M. Davis*, Dept. Cellular and Structural Biology, Univ. of Texas Hhsc. Ctr. at San Antonio, TX 78224-7762.

Depressant barbiturates suppress the stimulatory effects of the excitatory amino acids on neuronal activity and perhaps act preferentially on activities mediated by kainate receptors, but have little effect on NMDA-related receptors (Collins, G.G.S., Neuropharmacology, 26:167, 1987). To investigate this latter effect further, granule cell cell bodies were cultured from kainate-sensitive (SC) and kainate- insensitive (IC) rats. Cultures were preincubated in buffer or in buffer containing Na+ pentobarbital for 10 min before the addition of kainate or NMDA (125-1000 mM). One minute later the reaction was stopped, and cyclic GMP was determined by RIA. Pentobarbital (200 mM) markedly suppressed the elevations of cyclic GMP induced by 25-75 M kainate to near the control value while the 1000 M kainate was only partially suppressed. The elevation of cyclic GMP produced by 25 M kainate was significantly reduced by dosages of pentobarbital as low as 100 M, but further dosages remained to be tested. Similar effects of pentobarbital were observed on NMDA-induced elevations of cyclic GMP. These results demonstrate that the drug effects are mediated by excitatory amino acid-mediated neuronal stimulation, but, as yet, suggest no selectivity for receptor subtype. Supported by NIDA # 80755.
451.1 PHARMACOLOGICAL PROFILE OF SUBMAXIMAL CORTEX-ELECTROSHOCK KINDLING: LACK OF INVOLVEMENT OF NMDA RECEPTORS IN Harris, CC Garske, FP Cogen, LR Freedman and CC Palmus, Fisons Pharm, Div. R & D, Box 1710, Rochester, NY 14603. Rats receiving repeated submaximal cortical electrical stimulation (60Hz, 2mA x 2sec) exhibited a progression of seizures reminiscent of amygdala kindling (Rupfenhuber, 1989). Epilepsy 8: 1, 71-7.) The time course and pharmacology of "cortical kindling" have been examined. Rats were stimulated 1 & 2 hr post oral dosing for 5 days, followed by a 2 day wash out and retest. Vehicle-treated rats reached Stage IV-V seizures after 5 days. Established seizures were blocked by phenobarbital (30 mg/kg), cinnarizine (100 mg/kg) or the NMDA antagonist MK-801 (0.33 mg/kg). Seizure progression during kindling was slowed by MK-801, phenobarbital, valproate (704 mg/kg) or clonazepam (1 mg/kg). However, seizure severity judgment marked by the first test after wash-out of MK-801 or phenobarbital, approximating that in vehicle-treated rats; wash-out of MK-801 revealed only a slight increase in severity. Kindling was not slowed by phenytoin, remacemide or FPL 13950 (60, 60 & 66 mg/kg, respectively); the latter two blocks NMDA-induced seizures in mice (Edwards-57 & 30 mg/kg). Established seizures also were not blocked by phenytoin, remacemide or FPL 13950 (200 mg/kg). Coronal kindling differs pharmacologically from MES or PTE seizures, and some drugs thought to retard kindling (including NMDA antagonists) may only mask its expression.

451.2 KINDLING INCREASES THE APV AND CNQX SENSITIVE COMPONENTS OF SYNAPTIC RESPONSES IN BASOLATERAL AMYGDALA (BLA). B.F. Rainnie, K.K. Asperdon, & P. Shinnick-Gallagher, Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, TX 77550. Kindling induces burst firing responses in the amygdala. The purpose of these experiments was to analyse the subthreshold synaptic responses underlying this bursting behaviour. Stimulation of the stria terminals (ST) or lateral amygdaloid nucleus (LA) evoked a monophasic waveform, consisting of an EPSP, a fast IPSP and a slow IPSP recorded at -60mV using intracellular recording techniques. The EPSP consisted of a fast (rise-time 10-90%: 6 ± 1mV) CNQX (10uM) sensitive component which is enhanced with membrane hyperpolarisation. In the presence of CNQX (10uM) a slow (rise-time 19 ± 1.5mV) APV (10uM) sensitive component is revealed which is reduced with membrane hyperpolarisation. Following kindling, increases in both glutamatergic components of the EPSP are observed. In ShM APV, stimulus intensities evoking a CNQX sensitive EPSP (15 ± 3.5mV) in control (C) neurones cause burst firing in kindled (K) neurones. Similarly, in CNQX (10uM) significant increases in the APV sensitive component (3.0 ± 0.3mV, K: 1.4 ± 0.2mV, C: p = 0.012, 70mV) were recorded at lower stimulus intensities (mean: 24V, C: 18V). This APV sensitive EPSP is reduced with membrane hyperpolarisation. At all membrane potentials tested, no significant changes in membrane input resistance were observed following kindling. In addition, firing frequency in response to a transient (100ms) depolarising current step (0.4-6a) show no significant difference (6.6 ± 0.7, C: 4.1 ± 0.6, K). Furthermore, APV sensitive EPSPs, recorded in CNQX and bicuculline, in control were smaller than those in kindled neurones. These data suggest that changes in voltage dependent Mg2+ block, postsynaptic conductance, firing frequency and/or loss of GABAergic shunt conductance could not account for the enhanced glutamatergic EPSPs. However, the data could be explained by pre- and post-synaptic changes in glutamatergic transmission. Supported by NS 24643.

451.3 THE NMDA-ANTAGONIST, MK-801, REDUCES THE AMOUNT OF GRANULAR CELL HILUS BLOCK PRODUCED BY PERFORMANT KINDLING. MJ Gilbert, NI Technology Services Corp, RTP, NC, 27709. Electrical kindling of the perforant path or dentate hilus produces a reduction in inhibition as indexed by the paired pulse stimulation. Antagonists of the NMDA-receptor subtype of glutamate delay the development of kindling. The present experiment examined the effects of MK-801 on the development of enhanced inhibition in saline- and MK-801-treated rats during kindling of the perforant path. Kindling was induced by daily delivery of stimulus trains (2x 60 Hz 10 pulse duration, 800 µA peak to peak) to the perforant path for 30 min following 0.1 or 1.0 mg/kg MK-801 (ip). Pairs of stimulus pulses were delivered at 8 interpulse intervals (IP) ranging from 200 to 5 ms. The intensity of the conditioning pulse X was set at approximately 75% of the asymptotic population spike for a given subject. Over the course of kindling development, this intensity was adjusted to match the size of the population spike of saline. As previously reported, MK-801 increased AD thresholds and the number of sessions required to reach Stage 5 seizures. Pairs of inhibitory pulse functions (baseline), 24 hr after the 1st, 2nd, 6th and 15th AD indicated that MK-801 reduced the amount of inhibition that developed during kindling of control subjects. The effects of MK-801 were most pronounced from 150 ms which result in paired pulse facilitation under baseline conditions. The enhancement of a late period of inhibition occurring between 200 and 150 ms was also decreased by MK-801. Thus, in addition to the suppression of potentiation in excitatory projection pathways that accompanies kindling, NMDA-antagonists may also suppress potentiation of inhibitory circuits.

451.4 NMDA RECEPTOR BLOCKADE PREVENTS LENGTHENING OF AFTERDISCHARGES AND LOSS OF PAIRED PULSE INHIBITION INDUCED BY RAPIDLY REPEATING EPSCAPAL SEIZURES (RRHS). METHOD OF KINDLING. Jaldeep Kapoor1 and Eric W. Lothman2, Dept. of Neurology, Medical College of Virginia, Richmond, VA 23298 and Dept. of Neurology and Neuroscience, University of Virginia, Charlottesville, VA 22903. These experiments examine the role of NMDA receptor activation in the lengthening of afterdischarges observed in rapidly recurring hippocampal seizures (RRHS) method of kindling. We have previously shown that delivery of RRHS to awake rats causes a rapid kindling and that BWS in awake and urethane anaesthetized rats causes progressive lengthening of afterdischarges and a loss of paired pulse inhibition. Paired pulse inhibition is dominantly GABA mediated. Pretreatment with NMDA receptor antagonists Ketamine or MK801 prevented 8h induced lengthening of afterdischarges. Pretreatment with MK801 in a dose of 4mg/kg simultaneously prevented lengthening of afterdischarges and loss of paired pulse inhibition. The mechanisms behind kindling are unknown, however theoretical discussions of its pathophysiology focus on the balance between excitation and inhibition. Current and previously reported experiments demonstrate that NMDA receptor activation results in diminution of GABA mediated paired pulse inhibition which in turn mediates prolongation of afterdischarges observed in RRHS method of kindling.
545.2

The inhibitory neurotransmitter GABA appears particularly sensitive to amygdaloid epileptogenesis. The present study was designed to investigate the in vivo kindling effects on GABA-immunoreactive neurons of the lateral and basolateral amygdaloid nucleus in the rat. Male Sprague-Dawley rats were anesthetized and implanted stereotaxically with bipolar stimulating electrodes in the basolateral nucleus. One week later, the amygdaloid neurons were electrophysiologically characterized and lesioned with cathodal pulses of 1 ms, 500 nA applied at 60 Hz for 1 sec during the last 3.5 stage 5 seizures. The control group (N=4) consisted of non-stimulated implanted and unimplanted rats. Kindled rats developed generalized seizures after an average of 14 days (range: 9-16). Histological recovery of the electrode tips indicated that placements were in or near the basolateral complex for all subjects. There was no difference between the parietal and occipital regions of the brain using avidin-biotin peroxidase immunohistochemical techniques. Within the lateral and basolateral complex, discrete brain sections (2,12, 2,26, 3,14 and 3,60 mm from bregma, Paxinos & Watson, 1986) were chosen for quantification. The results indicate that, in comparison to controls, fully kindled animals showed a significant decrease in total number of GABA-IR neurons in the amygdala (ANOVA, F<.05). Furthermore, GABA-IR neurons throughout each of the four coronal amygdala planes were reduced in kindled animals (p < .05). The present data suggest that electrophysiological characterization or kindling will increase the number of GABA neurons in the amygdala which may ultimately contribute to amygdaloid epileptogenesis (Gean et al., 1989).

(Supported by NS24643 and DA07078)

545.7
REGIONAL ANALYSIS OF GABA-STIMULATED CHLORIDE FLUX IN AMYGDALA KINDLED RATS. R.J. Tierz and T.H. Chu. Dept. of Pharmacology, Medical College of Ohio, Toledo, OH 43699.

GABA-stimulated chloride flux was evaluated in 4 brain regions (cortex, CTX; hippocampus, HIP; midbrain/brainstem, MBR and cerebellum; CK) of amygdala kindled rats. Male rats (n=16) were stimulated 2 days after the last stage 5 seizure, were sacrificed at 15 min before and immediately after the last stage 5 seizures were elicited. Control rats (n=17) were sham kindled. Microssacs, prepared from 4 pooled kindled or control rats after the last stage 5, were placed for 5 sec in 15 ml of a medium where the GABA stimulation, were incubated at 15 min at 30°C. GABA (10 or 50 μM) and Cl<sup>-</sup> (0.4 μM) were added followed in 5 sec by rapid filtration and 2 buffer washes. Basal flux (μCi/mg protein) differed between regions but not between kindled and control groups (CTX, 17.7±9 vs 17.6±1; HIP 21.5±1.1 vs 20.6±1.1; MBR, 30.6±1.8 vs 30.2±1.3; CK 23.4±0.8 vs 26.5±1.0). There were no significant differences in net GABA-stimulated chloride flux in CTX and HIP only at the lower GABA concentration. The decrease in flux in CK (9.7±7 vs 6.4±1) was not significant. There was no difference in MBR (3.1±2 vs 2.9±2). There was no change in any brain region in the ability of 1 μM midazolom to stimulate 10 μM GABA-mediated flux. These results indicate long-lasting regionally specific changes in GABA complex function following amygdala kindling. The findings contrast those of decreased GABA-stimulated flux in brainstem of entorhinal-kindled rats and support those of decreased GABA complex function with kindling. Supported by grants S70-RR05705 and R01-DA04075.

545.9
NORADRENERGIC COMPONENTS OF FOREBRAIN KINDLING: THE ROLE OF AMYGDALOID NOREPINEPHINE. I. Nierenberg, CD Applegate and H. Burchfield. Comprehensive Epilepsy Program, University of Rochester School of Medicine, Rochester, NY 14642.

Forebrain deplation of norepinephrine (NE) facilitates kindling seizure development by reducing the number of trials spent in the early phase of kindling (seizure stages 1-2). We have hypothesized that this result indicates the existence of a discrete, NE-dependent transition from early to later phases of kindling (stages 3-5) kindling phases (Burchfield & Applegate. Neurosci. Biobehav. Rev. 13:1989). In this study we have attempted to simulate this transition by administering injections of NE (n=10) or saline (n=9) to rats, which facilitate this transition. Rats were injected bilaterally into the amygdala with 6-hydroxydopamine (6-OHDA, 10 μg in 1 μl) prior to the kindling lesioned amygdala nucleus (n=6). Amygdala-kindled rats with 6-OHDA lesions showed 40% reduction in the number of early, stage 1-2 (m<sub>2</sub> = 14.3±5.7) and later, stage 3-5 (m<sub>2</sub> = 14.3±5.7) seizures relative to unlesioned controls (m<sub>2</sub> = 22.0±3.9). These data support the involvement of amygdaloid NE in the kindling of forebrain structures. Ongoing experiments are being conducted to confirm whether the role of the amygdala is unique in this context or whether it is part of a limbic "loop" which modulates the NE-dependent transition from early to later stages of kindled seizure development.

545.10
AMYGDALA KINDLED SEIZURES AND BRAIN A<sub>1</sub> ADENOSINE RECEPTORS IN RATS. S.M. Anderson and D.D. Walczak,- Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20077-5100.

In order to understand the development of post-traumatic epilepsy we are assessing changes in brain neurochemistry during various stages of seizure promotion using an experimental post-traumatic kindling model. The anticonvulsant properties of adenosine and evidence that upregulation of adenosine receptors is accompanied by reduced sensitivity to a variety of anticonvulsants has prompted us to investigate the relationship between A<sub>1</sub> adenosine receptor and seizures. Male Sprague-Dawley rats were kindled from a biphal electrode implant placed in the left amygdala. Electrical stimulation that began once every 24 hrs until either a second consecutive stage 3 or three successive stage 5 seizures were achieved. EEG was recorded from the electrodes and the corresponding cerebral regions (n=10) or saline (n=9) were injected (n=11.5±1.0). Amygdala-6-OHDA also resulted in a reduced number of stage 1-2 trials in septal nucleus-kindled rats (m<sub>2</sub> = 14.3±4.7) relative to unlesioned controls (m<sub>2</sub> = 22.0±3.9). These data suggest an involvement of amygdaloid NE in the kindling of forebrain structures. Ongoing experiments are being conducted to confirm whether the role of the amygdala is unique in this context or whether it is part of a limbic "loop" which modulates the NE-dependent transition from early to later stages of kindled seizure development.
452.11

EPILEPSY:

Potential perforant

Neurology, 452.13

unilateral HIPP in the
corticostriatal field (ECS)
occurred during ECS
stimulation on the left
HIPP. This indicates that
the seizures are caused
by a direct activation of
the HIPP.

These rats were
implanted with electrodes
in the HIPP and the
entorhinal cortex. The
electrical stimulation
was performed on the left
HIPP, and the responses
were recorded in the
dorsal and ventral parts
of the hippocampus.

The results indicate
that ECS stimulation
causes a significant
increase in the
membrane potential of
the CA1 region of the
hippocampus. This
increase is associated
with a decrease in the
seizure threshold. The
seizure threshold was
reduced by ECS
stimulation, and the
response was prolonged
by ECS stimulation.

In conclusion, the
results suggest that
ECS stimulation
induces a sensitivity to
electrical stimulation in
the HIPP, which
contributes to the
development of
epileptic seizures.

452.12

KINDLING IN RATS: EVALUATION OF A HIGHLY SENSITIVE
dLOCUS IN THE POSTERIOR PART OF THE HIPPOCAMPUS.
F. Lüscher, U. Wahnschaffe* and D. Rossack

The pyriform cortex is
important in the
development of
epileptic seizures. Its
role in the
kindling process is
well documented.

This study investigated
the role of the pyriform
cortex in the
kindling process. The
rats were implanted
with deep brain
stimulation electrodes,
and the responses were
recorded in the HIPP
and the entorhinal
cortex. The ECS
stimulation was
performed on the left
HIPP, and the
responses were
recorded in the
dorsal and ventral parts
of the hippocampus.

The results indicate
that ECS stimulation
causes a significant
increase in the
membrane potential of
the CA1 region of the
hippocampus. This
increase is associated
with a decrease in the
seizure threshold. The
seizure threshold was
reduced by ECS
stimulation, and the
response was prolonged
by ECS stimulation.

In conclusion, the
results suggest that
ECS stimulation
induces a sensitivity to
electrical stimulation in
the HIPP, which
contributes to the
development of
epileptic seizures.
453.1
PREFERENTIAL EXPRESSION OF SUPEROXIDE DISMUTASE GENE IN THE NEUROMELANIN-PIGMENTED NEURONS OF THE SUBSTANTIA NIGRA: IMPLICATIONS FOR PARKINSON'S DISEASE.

Zhang P-T, Ceballos* (1), Hirsh E, Lafon M*, Sinet P M* (1), Agid Y and P. Froix-Aud

INSERM U 289, Hôpital de la Salpêtrière 75013 PARIS, (1) URA CNRS 1335 Laboratoire de Génétique des Maladies Neurodégénératives, INRA URA 1146, Laboratoire de Génétique des Maladies Neurodégénératives, Université Paris, France.

The dopaminergic neurons primarily affected in Parkinson's disease are the melanized neurons of the substantia nigra pars compacta. The involvement of oxygen free radicals has been considered as a potential cytotoxic cause of cell death. The present study searched for specific characteristics of these two cell systems in these nigral neurons. Superoxide dismutase (SOD) which catalyses the conversion of superoxide radicals to hydrogen peroxide was examined.

The gene expression of CuZnSOD (CuZnSOD) in the substantia nigra pars compacta was studied at cellular level in human mesencephalon post-mortem, by in situ hybridization using a 35-S-labelled cDNA probe logarithmic to human CuZnSOD mRNA. Labelled cells were densely distributed in the substantia nigra pars compacta, some cells were present in the ventral tegmental area. 95% of the positively hybridized nigral cells were the large neuromelanin-containing cells, indicating CuZnSOD gene is preferentially expressed in the pigmented dopaminergic neurons. The high levels of CuZnSOD transcripts suggest the biochemical pathways leading to toxic oxygen species formation are active thus requiring a high CuZnSOD protein to facilitate removal of the toxic radicals. Alternatively, a high CuZnSOD activity might contribute to the neurodegenerative process itself.

The nigral melanized neurons represent a subset of dopaminergic cells with respect to their oxygen defense system. This may account for their preferential vulnerability in Parkinson's disease.

453.3
CEREBRAL METABOLIC CHANGES DURING THE 'ON-OFF' PHENOMENON IN PARKINSON'S DISEASE: A PET STUDY WITH APOMPHORINE.


Long-term levodopa treatment of Parkinson's disease (PD) provokes disabling motor fluctuations ('off'-on phenomenon). We measured regional cerebral glucose utilization during the "on" and "off" state using the [18F]-fluorodeoxyglucose (FDG) method and positron emission tomography (PET) in 5 right-handed non-demented PD patients (4 males, 1 female; mean age ± SD: 60.2 ± 7.5 yrs; duration of disease: 12.2 ± 2.9 yrs; duration of "on-off" phenomenon: 4.1 ± 2.3 yrs). Patients had severe akinnesia with no tremor during the "off" phase, and no or few dyskinesia during the "on" phase. Two PET scans were performed 48 hrs apart. Antiparkinsonian medications were discontinued the evening before each PET day. 15 min prior to FDG injection, patients (while in an "off" phase) received s.c. amphetamine (AP0, a potent dopaminergic agonist, or the vehicle. The APO dosage (3-6 mg) was able to relieve akinnesia but less than 15 min, and for a duration of about 1 hr. Patients were taking a peripheral dopaminergic agonist, dopiperidone (60 mg/day orally), to block APO effects on cerebral blood vessels. Our results reveal that APO tends to reduce glucose utilization in the whole cerebral cortex (-8.8%; p = 0.08, NS; ANOVA) and in most of the 12 regions analyzed (p = 0.08 to 0.19; significance only in the temporal cortex, p = 0.05, and the cerebellum, v < 0.01).

453.5
STEP FORCE CHANGES IN PARKINSONIAN PATIENTS. F. Muller* and C.f. Stelmach, Motor Behavior Laboratory, University of Wisconsin, Madison, WI 53706.

Force production is an essential part of all motor acts. Previously, Parkinson's Disease patients (PD) have been shown to produce accurate isometric forces (Stelmach, G. et al., 1988), but with a high variability (Stelmach, G., et al., 1989). Less interest had been shown for force termination problems in PDs. Eight PDs with a mean age of 60 ± 10 years controlled performed step changes of isometric pinch and elbow flexion forces. Starting from 0% of maximum voluntary contraction level, the task was to increase, and to decrease the force level by 15% and 30% respectively, as accurately as possible. The results showed that absolute peak force levels differed among and between groups. Reaction times indicated a slugging for force release compared to force increase tasks. The accuracy requirements affected finger and elbow joint forces differently. Movements taken a longer duration for release compared to complete release for groups and conditions, while the pinch force increase for normals used the same across pinch force amplitudes. For the peak force, no subject of the step changes (first derivative) confirmed that the ability of force impulse scaling was impaired in PDs. The unusual accuracy requirements required, however, that normals and PDs adapted a movement time scaling strategy.

453.8
TEMPORAL-SPATIAL IRREGULARITIES IN PARKINSONIAN INDEX FINGER AGONIST/ANTAGONIST MUSCLE ACTIVITY. C.J. Huner* and J.H. Abbs, Dept. of Neurology, Speech and Motor Control Lab, Walterman Center, Univ. of Wisconsin, Madison, WI 53706.

Normal rapid, goal-directed limb movements are accomplished by regulating the intensity and timing of triphasic antagonistically acting muscles. A striking feature of Parkinson's disease (PD) is abnormally slow movements (bradykinesia). Temporal and amplitude scaling of the triphasic muscle activity have been proposed as the pathological substrate for this clinical sign in the limbs. The intent of this study was to examine the pathophysiology of bradykinesia movements not typically mediated by the classical triphasic neural control strategy.

Index finger extension/flexion reversal movements of variable extent and intramuscular EMG activity and amplitude scaling were studied in 6 normal subjects and 3 PDs. Significant bradykinesia movements not typically mediated by the classical triphasic neural control strategy.

A linear relationship between peak velocity and movement amplitude was found in normals. In contrast, PD responses were variable; most were outside the normal 95% confidence interval. Possible factors in the various points along index finger extension trajectories characterized the bradykinetic movements, but could not be explained by gross EMG burst patterns. However, temporal-spatial irregularities in PD agonist/antagonist muscle activity may underly the abnormal phase plane relationships. (NIH Grant NS-133274)
453.7 ANTICIPATORY AND FEEDBACK POSTURAL RESPONSES IN PARKINSON'S DISEASE. S.L. Glatt, M.E. Melnick, W. Kolter, B. Hasenfeld, J. Nash, J. Redford, G. Jayaraman. Departments of Neurology, Physical Therapy, and Rehabilitation Medicine, University of Kansas Medical Center, Kansas City, KS 66103.

The mechanism underlying postural instability in Parkinson's disease is not fully understood. In order to understand postural responses, we studied the anticipatory postural response and feedback-related responses by measuring the movement of the center of pressure (CP) with a forward lifting of a 0.5 kg bar, in 37 PD patients: 16 stage 1 (PD1), 14 stage 2 (PD2) and 7 stage 3 (PD3) compared to 9 elderly (EC) and 15 young controls (YC). Maximal forward displacement, which reflects the deficit in anticipatory responses, was measured for both PD1 of 1.60 cm, PD2 1.96, PD3 2.91, EC 1.60 and YC 1.87. Averaged position of the CP, which is under feedback control, was for PD1 0.75, PD2 0.85, PD3 1.93, YC 1.19, and EC 0.75. There were differences (p < .01) between PD 3 and all other groups in the anticipatory responses and between PD3 and all except YC in the feedback response. Maximal mean CP position for PD3 (2.91 cm) was similar to the biomechanical calculation of CP for the barlift posture with arms outstretched (3.2 cm) without any compensatory responses. There was more impairment in anticipatory than feedback responses. We suggest that abnormalities in anticipatory postural responses are the cause of postural instability and falls in PD.

453.8 COGNITIVE AND MOTOR SEQUENCING IN PARKINSON'S DISEASE. W.K. Beatty and N. Monsen. Neuropsychiatric Research Institute, Fargo, ND 58107.

Previous reports indicate that patients with Parkinson's disease (PD) may have particular difficulties on tasks that require accurate sequencing in time or space. However, in all previous studies, the apparent sequencing deficit could also be attributed to impairment in memory, visual perception or motor function that occurs in PD.

In the present study we administered a very simple untimed test of cognitive sequencing (arranging pictures of highly familiar events in order) and a version of the Luria 3-step task of motor sequencing to 25 patients with idiopathic PD and 25 age- and education-matched normal controls. Both demented and non-demented PD patients displayed impairments on both sequencing tasks. The extent of the patients' impairments in cognitive and motor sequencing were positively correlated, and the severity of deficits on both sequencing tasks was related to performance on the Wisconsin Card Sorting Test, but not to neurologic measures of disease severity. These results demonstrate the existence of a cognitive sequencing deficit in PD, and suggested that in PD both cognitive and motor sequencing difficulties may be related to dysfunction of circuits that involve the frontal lobes.

453.9 MOVEMENT SEQUENCING IN PARKINSON'S DISEASE. E. A. Roy, J. Saint-Cyr, A. Taylor & A. Lang*. Movement Disorders Clinic and Department of Psychology, Toronto Western Hospital, Toronto, Ontario, Canada. M5T 2S8.

The purpose of this study was to examine movement sequencing in Parkinson's disease (PD). Fifteen PD patients and five age-matched normals were required to learn a sequence of 3 or 4 hand movements on a sequencing bar. Each trial involved two phases, a perceptual phase in which pictures depicting the movements were present and, if the patient performed correctly, a memory phase in which he attempted to perform the sequence from memory without the aid of the pictures. The criterion for learning was 5 consecutive correct trials with no errors. Videdom performances were examined in terms of trials to reach criterion, errors (sequencing errors and perseverations), total response time and its components, time for each response element and time between consecutive responses. There were no group differences in trials to criterion or errors, but marked differences in response timing were apparent. Total response time was significantly longer for the PD patients which was due to both increased time to make each response as well as increased interresponse times. The relative timing reflected in the proportion of the total time spent in each component of the sequence was also significantly different for the two groups. The implications of these findings for understanding the movement sequencing impairments in PD are discussed. (Supported by a grant from The Parkinson's Foundation of Canada.)


Groups of patients with idiopathic Parkinson's disease, either medicated or unmedicated, were compared alongside a large sample of normal controls on a computerised battery of tests designed to investigate the cognitive processes involved in 'planning' and 'sequential memory' tasks. Based on the 'Tower of London' task, a group of medicated patients were shown to be impaired in the amount of time spent thinking about (planning) the solution to each problem. Additionally, an impaired performance in terms of the number of moves required to reach a solution on this test was also evident in those patients 'later in the course' of the disease and was accompanied by a deficit in associated tests of spatial working memory and spatial span. In contrast, a group of patients who were unmedicated and 'early in the course' of the disease were unimpaired in all respects compared to normal controls.

These data are compared to those from a group of young neurosurgical patients with localised excisions of the frontal lobes and are discussed in terms of the progressive nature of the cognitive deficit in Parkinson's disease.

453.11 PARKINSON'S DISEASE AND FLUCTUATIONS IN L-DOPA DO NOT IMPAIR EXPONENTIAL VISUAL LEARNING. J. Doggen, D. Contant, A. Pelletier, G. Gouillette, Ecole de Psychologie, Universite Laval, Ste-Foy, QC, Canada, G1K 7P4.

Recognition tasks have shown to be very useful for studying memory functions in Parkinson's disease (PD) because they do not require the participation of the motor system. Such paradigms was employed in the present study to evaluate the effects of PD and of fluctuations in levels of L-dopa on explicit visual learning. The performance of 10 non-demented PD patients, both during fluctuations in L-dopa stimulated and unstimulated states (i.e., approximately 16 hours after the last drug intake) was compared to that of healthy controls on the visual paired associates subset of the Wechsler Memory Scale-Revised. A new version of this task was also created to eliminate any practice effect between the two testing sessions. These tasks were administered on separate days in a counterbalanced order. The results of the two tasks were parallel and indicate that the decrease in the level of l-dopa did not affect their learning ability, although it produced significant changes in L-dopa levels. These results suggest that Parkinson's disease and variations in levels of L-dopa do not impair explicit visual learning.


We previously reported that the extraneural administration of L-dopa at a dose of 5-10 mg daily, has been efficacious for delaying the need for the MAO inhibitor. In rats and mice, single doses of 2 mg/kg of 1-deprenyl selectively and completely metabolizes monoamine oxidase (MAO), with no significant inhibition of MAO-A activity. However, in the present study with 1-deprenyl and other selective, irreversible MAO-B inhibitors, (M7, 72145, AG 1138) the daily administration of doses (0.2 to 2.0 mg/kg) to mice and rats for up to 4 weeks led to a loss in selectivity of MAO-B inhibition, with extensive inhibition of MAO-A occurring both in the brain and periphery. Interestingly, when rodents were given 2 mg/kg of clorgyline, a highly selective MAO-A inhibitor, for the same length of time no crossover to B inhibition was seen. It is possible that the administration of 1-deprenyl to PD patients also leads to MAO-A inhibition, raising the possibility that these patients may be susceptible to hypertension after ingesting foods containing tyramine (the cheese effect). This correlates with other work done on the effects of daily doses of 1-deprenyl increase the pressor effects to a tyramine challenge in idiopathic Parkinson's disease. Moreover, 1-deprenyl is extensively metabolized to L-methamphetamine and 2-methoxyamphetamine, common CNS stimulants. All of these observations suggest that the effects of 1-deprenyl may not be due solely to its inhibition of MAO-B activity.
(1-deprenyl and dopamine transmission: electrophysiological recordings in the rat caudate nucleus. I.A. Paterson, M.D. Berry and A.V. Horio. Neuropsychiatric Research Unit, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

(-)deprenyl is a specific monoamine oxidase type B (MAO-B) inhibitor which has been used as an adjunct to L-DOPA therapy because it lowered the daily requirement for L-DOPA and its side effects. Recently, it has been shown that (-)deprenyl delays the progression of Parkinson's disease in the earlier stages of the disease. It has been assumed that (-)deprenyl acts mainly by inhibiting the oxidative deamination of dopamine in the central nervous system (CNS) and hence, acute administration of (-)deprenyl does not affect DA metabolism.

We propose that the acute action of (-)deprenyl may involve 2-phenylethylamine (PE), an intermediate metabolite of DA and PE may coexist with DA in the nigro-striatal projection as 6-hydroxydopamine lesions or electrical stimulation of the nigra alters its levels in the striatum. PE is not stored in receptor-sensitve granules and is released by diffusion down a steady-state concentration gradient between the terminal and glial cells where it is metabolized by MAO-B. In rats, (-)deprenyl (0.54 mg kg\(^{-1}\)) potentiates caudate neuron responses to DA agonists, an effect that is similar to the actions of PE (Paterson et al., unpublished data). (-)Deprenyl and dopamine transmission: electrophysiological recordings in rat caudate nucleus, this meeting. Supported by the Parkinson Foundation of Canada and the MRC of Canada.

Supported by Saskatchewan Health and Saskatchewan Health Research Board.

1110 degenerative disease—Parkinson's: humans and treatment Thursday am

1111 degenerative disease—Parkinson's: humans and treatment Thursday am

1112 degenerative disease—Parkinson's: humans and treatment Thursday am

Society for Neuroscience abstracts, volume 16, 1990

453.13
(1-deprenyl and dopamine transmission: electrophysiological recordings in the rat caudate nucleus. I.A. Paterson, M.D. Berry and A.V. Horio. Neuropsychiatric Research Unit, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

(-)deprenyl is a specific monoamine oxidase type B (MAO-B) inhibitor which has been used as an adjunct to L-DOPA therapy because it lowered the daily requirement for L-DOPA and its side effects. Recently, it has been shown that (-)deprenyl delays the progression of Parkinson's disease in the earlier stages of the disease. It has been assumed that (-)deprenyl acts mainly by inhibiting the oxidative deamination of dopamine in the central nervous system (CNS) and hence, acute administration of (-)deprenyl does not affect DA metabolism.

We propose that the acute action of (-)deprenyl may involve 2-phenylethylamine (PE), an intermediate metabolite of DA and PE may coexist with DA in the nigro-striatal projection as 6-hydroxydopamine lesions or electrical stimulation of the nigra alters its levels in the striatum. PE is not stored in receptor-sensitve granules and is released by diffusion down a steady-state concentration gradient between the terminal and glial cells where it is metabolized by MAO-B. In rats, (-)deprenyl (0.54 mg kg\(^{-1}\)) potentiates caudate neuron responses to DA agonists, an effect that is similar to the actions of PE (Paterson et al., unpublished data). (-)Deprenyl and dopamine transmission: electrophysiological recordings in rat caudate nucleus, this meeting. Supported by the Parkinson Foundation of Canada and the MRC of Canada.

Supported by Saskatchewan Health and Saskatchewan Health Research Board.

1110 degenerative disease—Parkinson's: humans and treatment Thursday am

1111 degenerative disease—Parkinson's: humans and treatment Thursday am

1112 degenerative disease—Parkinson's: humans and treatment Thursday am

Society for Neuroscience abstracts, volume 16, 1990

453.15

Rats with unilateral 6-hydroxydopamine lesions were treated with L-DOPA (10 or 20 mg/kg IP) plus Carbidopa (1:0 or 2:0 mg/kg IP). The effects of L-DOPA on Dopaminergic neuron: a novel treatment for Parkinson's disease. Robert J. Payne Department of Psychiatry S.U.N.Y. and VA Medical Center, Syracuse, NY 13210.

Rats with unilateral 6-hydroxydopamine lesions were treated with L-DOPA (10 or 20 mg/kg IP) plus Carbidopa (1:0 or 2:0 mg/kg IP). The effects of L-DOPA on Dopaminergic neuron: a novel treatment for Parkinson's disease. Robert J. Payne Department of Psychiatry S.U.N.Y. and VA Medical Center, Syracuse, NY 13210.

Rats with unilateral 6-hydroxydopamine lesions were treated with L-DOPA (10 or 20 mg/kg IP) plus Carbidopa (1:0 or 2:0 mg/kg IP). The effects of L-DOPA on Dopaminergic neuron: a novel treatment for Parkinson's disease. Robert J. Payne Department of Psychiatry S.U.N.Y. and VA Medical Center, Syracuse, NY 13210.

Rats with unilateral 6-hydroxydopamine lesions were treated with L-DOPA (10 or 20 mg/kg IP) plus Carbidopa (1:0 or 2:0 mg/kg IP). The effects of L-DOPA on Dopaminergic neuron: a novel treatment for Parkinson's disease. Robert J. Payne Department of Psychiatry S.U.N.Y. and VA Medical Center, Syracuse, NY 13210.

Rats with unilateral 6-hydroxydopamine lesions were treated with L-DOPA (10 or 20 mg/kg IP) plus Carbidopa (1:0 or 2:0 mg/kg IP). The effects of L-DOPA on Dopaminergic neuron: a novel treatment for Parkinson's disease. Robert J. Payne Department of Psychiatry S.U.N.Y. and VA Medical Center, Syracuse, NY 13210.

Rats with unilateral 6-hydroxydopamine lesions were treated with L-DOPA (10 or 20 mg/kg IP) plus Carbidopa (1:0 or 2:0 mg/kg IP). The effects of L-DOPA on Dopaminergic neuron: a novel treatment for Parkinson's disease. Robert J. Payne Department of Psychiatry S.U.N.Y. and VA Medical Center, Syracuse, NY 13210.

Rats with unilateral 6-hydroxydopamine lesions were treated with L-DOPA (10 or 20 mg/kg IP) plus Carbidopa (1:0 or 2:0 mg/kg IP). The effects of L-DOPA on Dopaminergic neuron: a novel treatment for Parkinson's disease. Robert J. Payne Department of Psychiatry S.U.N.Y. and VA Medical Center, Syracuse, NY 13210.

Rats with unilateral 6-hydroxydopamine lesions were treated with L-DOPA (10 or 20 mg/kg IP) plus Carbidopa (1:0 or 2:0 mg/kg IP). The effects of L-DOPA on Dopaminergic neuron: a novel treatment for Parkinson's disease. Robert J. Payne Department of Psychiatry S.U.N.Y. and VA Medical Center, Syracuse, NY 13210.
454.1

**Effects of methamphetamine and 
ME-801 on dopamine in the striatum of awake rats as measured by in vivo brain microdialysis.**


Although the NMDA receptor antagonist ME-801 attenuates methamphetamine (MA)-induced striatal dopamine (DA) neurotoxicity, the mechanism underlying this protection is unknown. We used in vivo microdialysis in awake rats to determine the effects of ME-801 and MA on the levels of extracellular striatal DA (and metabolites) and behavior. A single injection of MA (6.25 mg/kg, sc) induced a prolonged increase (> 6 hrs) in extracellular DA (an EIC peak response), reduced basal levels, and increased sniffing, circling, and locomotion. Given alone, ME-801 (0.5 mg/kg, ip) also produced an increase in extracellular DA, a peak response, shorter duration than that with MA, and increased sniffing and locomotion. In contrast to MA, ME-801 increased extracellular DA levels and prevented the circling response. ME-801 markedly attenuated the MA-induced DA increase and increased rather than decreased DOPAC. Despite its attenuation of MA-induced DA increases, ME-801 potentiated some components of MA-induced behaviors and increased their duration. These findings suggest that although ME-801 alone increases extracellular striatal DA, it reduces the MA-induced striatal DA overflow, which may help explain its attenuation of MA-induced dopaminergic terminal injury.

**NEUROTOXICITY: AMINO ACIDS**

454.2

**INTERACTIONS OF ME-801 WITH GLUTAMATE- AND METHAMPHETAMINE-EVOKED STRIATAL RELEASE.**


ME-801 effects on L-glutamate (GLU) and methamphetamine (METH)-evoked release of H- dopamine (DA) from striatal slices in untreated and rats treated with toxic doses of METH (5 mg/kg, 4 doses/2 hr intervals) were evaluated. With no METH present, 40 μM and 1 mM GLU evoked a H-DA release (5% or 1% of total H-DA stores [TS]) over 10 min, respectively) from striatal slices of untreated rats. GLU-evoked release was inhibited by ME-801>7CPG+ (IBO, 10-4). Two weeks after in vivo METH, GLU-evoked release (no Mg2+ present) was decreased. With 1.25 mM Mg2+ present, 10 μM GLU evoked a slight release of H-DA (2.1% TS) which was increased to 4.2% TS when 10 μM nonfenamine was present. Release increased 2.5% by 5 μM ME-801. With or without 1.25 mM Mg2+, 0.5 and 5 mM METH evoked a release of H-DA (7% and 20% TS over 10 min, respectively) which was additive with GLU-evoked release. ME-801 (5μM) did not affect METH-evoked H-DA release with or without Mg2+ present. These results indicate that ME-801 protection against METH toxicity is not via presynaptic inhibition of METH-evoked DA release. However, since presynaptic release of DA by METH and GLU is additive, ME-801 might reduce GLU-mediated DA release during METH exposure.

454.3

**EFFECTS OF HIGH-DOSE METHAMPHETAMINE (MA) ON NORPINEPHRINE (NE) AND DOPAMINE (DA) UPTAKE SITES IN RAT BRAIN MEASURED BY QUANTITATIVE AUTORADIOGRAPHY.**


It has been reported that high doses of MA cause neurotoxic effects on serotonin (5-HT) and DA neurons in brain. However, NE neurons have been thought to be spared from neurotoxic effects. In addition, the neurotoxic effect of MA on DA uptake sites have previously been examined only in striatum. We have examined the effects of MA on NE and DA uptake sites in rat brain by quantitative autoradiography using [3H]-noradrenaline (Tejani-Butt et al., this meeting) or [3H]-mazindol, respectively. Rats were treated with either MA (15mg/kg free base, n=10) or saline (n=10) given i.p. every 6 hrs for 3 days and killed 7 days after the last dose. Areas examined were from brain slices taken at plates 30 and 57 of the atlas of Paxinos and Watson (1986) for NE uptake sites and plates 13 and 37 for DA uptake sites. MA caused significant reductions (17-33%) in terminal and lateral amygdaloid nuclei and dorsomedial hypothalamus. No reductions were seen in any other area, including locus coeruleus, a cell body area. DA uptake sites were decreased by 23-53% in DA terminal areas - caudate putamen/nucleus accumbens and olfactory tubercle; in contrast, they were unaffected in DA cell body areas - ventral tegmental area and substantia nigra. We have previously shown that MA is neurotoxic in 5-HT terminal areas (Brain Res., 505:123, 1990). We conclude that MA may be neurotoxic to NE neurons in specific brain areas, and NE neurotoxicity is limited to terminal field areas reported by research funded by the Department of Veterans Affairs & USPHS grant DA 03517.

**BIPHASIC RELEASE OF DOPAMINE (DA) FROM CORPUS STRIATUM (CS) IN VITRO AND ELEVATION OF AMINO NEUROTRANSMITTER METABOLITES IN CEREBROSPINAL FLUID (CSF) OF PUSHPULL PERFUSED RATS BY AMINONIA, S. Kahanmghan, V.D. Ramirez, and R.V. Burgardt*, Dept. of Physiology, University of Illinois, Urbana, IL 61801.

Aminomethane (0.5-22mg/ml) releases dopamine (DA) and DOPAC in a biphasic manner from fragments of rat CS (4). The first phase reaches plate at 3min, when the DA release reaches 540±258, but not by inosintoxin (DA release, 530±209). It is postulated that the first phase is due to depolarization of DA neurons by the uptake carrier as reversal of transport, analogous to the action of amphetamine. Preliminary results show that intraperitoneal (ip) injection of 30mg/kg NAC produces 20 fold elevation in the CSF concentrations of DOPAC and 5-HIAA, which is push-pull perfused rats in the lateral ventricle. These high levels last for 2h and did not occur in control rats infused with ip saline. Ammonia concentrations above 1000ng are found in hepatic coma and the release of amine neurotransmitters may be responsible in the pathogenesis.
454.5


Methamphetamine (METH) administered to mice in repeated high doses selectively neurotoxic to dopaminergic neurons in the neonate. This neurotoxicity is dependent upon the METH dose. The extent of damage caused by a given dose of METH, which is assessed by decrement in striatal dopamine content, and tyrosine hydroxylase activity, varies among different strains of mice. For example, in the present study we found that C57 mice were substantially more sensitive to the neurotoxicity induced by METH than were CB6f1 mice; both strains came from Charles River Breeding Laboratories. A lesser but still significant difference was observed between two strains obtained from Jackson Laboratories; SWR/J mice were more sensitive to METH-induced neurotoxicity than were C57/J mice. In experiments done with neostriatal tissue slices obtained from C57/J mice, there were no pronounced differences in the capacity of METH to cause dopamine release. Our laboratory previously demonstrated that (100), a non-competitive NMDA receptor antagonist, was able to attenuate METH-induced neurotoxicity, suggesting that glutamate plays a role in mediating the damage. Interestingly, the C57/J mice are more sensitive to the SWR/J mice to the stimulation and lethal effects of the mexitlyanines, which are adenosine receptor antagonists. These strains also have different numbers of adenosine receptors. It is possible that variations in the interactions between or among dopamine/glutamate/adenosine may contribute to the observed differences in METH-induced neurotoxicity.

454.7

ROLE OF GANGLIOSIDES AND CA-M-dependent ENZYMES IN GLUTAMATE NEUROTOXICITY. H. Manev, A. Guidotti, R. Simoni, and E. Costa. PGIN, Georgetown University, Washington, DC 20007.

We have shown that neostriatal neuron cultures contain natural gangliosides (GM1, GTb) or by the application of their synthetic derivatives (LIGA4, LIGA20). (Pharmacol. Exp. Ther., 1990;22:341). We studied, in primary cultures of rat neostriatal, glycine receptor, and NMDA receptor antagonists. The influence of these gangliosidic on glutamate-induced: (a) activation of Ca-dependent cysteine proteinase (calpain) by glycine receptors with the high selective neurotoxicity of the neurotransmitters. We therefore translocated to the protein kinase C (PKC) by immunoblotting and 3H-phorbol ester binding. Calpain activity was dependent on the nsic turnover of Ca2+/-molar concentrations of Ca2+ (calpain 1), and on millimolar concentrations (calpain 2) in glial cultures. Glutamate induced a dose-dependent activation of calpain 1 and the translocation of PKC only in neuronal cultures. These changes lasted the removal of a toxic dose of glutamate from the culture and preceded neuronal death. Pretreatment with LIGA4 prevented the translocation of PKC and prolonged the activation of calpain 1, and neuronal death. In vitro experiments excluded a direct inhibitory action of gangliosides on calpain 1. The prolonged translocation of PKC may be operative in maintaining the increased calpain activity elicited by neurotoxic doses of glutamate.

454.8

OPTIMIZATION OF CONDITIONS FOR SEPARATION OF TEN TRYPTOPHAN METABOLITES BY RP-HPLC. C-Z. Chuang (1,2), F.A. Regan, Jr. (1,2) and C. Prasad (1,2). Lab. of Neurosciences (1), Pennington Biomedical Research Center; Departments of Pathology (2) and Medicine (3) (Endocrinology Section), LSU Medical School, New Orleans, LA 70112.

Tryptophan (TRP) hydroxylase and TRP oxygenase (TO) are two major pathways of TRP metabolism in brain. The TO pathway yields many interesting neuroactive metabolites: quinolinic acid, kynurenine acid, kynurene and 3-hydroxykynurene. Studies into the presence and the roles of these and other potential TRP metabolites (anthranilic acid, 3-hydroxyanthranilic, xanthurenic, quinolinic, and picolinic acids) in brain are limited by our inability to measure low levels of multiple metabolites in a single biological sample. Therefore, a RP-HPLC method for the separation of these metabolites has been developed by sequential optimization of mobile phase pH, triethylamine concentration and gradient elution. Resolution obtained is very good with an analysis time, including re-equilibration period, of less than 30 min. To our best knowledge this is the first RP-HPLC method that separates TRP and ten metabolites of TO pathway in a single chromatographic run.

454.10

IS TAUrine INVOLVED IN THE PATHOGENESIS OF HEPATIC ENCEPHALOPATHY? S.S. Olg, P. Saranannagi and U. Uvayn, Department of Neurology, University of Tampere, Box 607, SF-33101 Tampere, Finland, and Medical Research Centre, Polish Academy of Sciences, 00-784 Warsaw, Poland.

The possible role of taurine in hepatic encephalopathy (HE) was studied with rats injected with thioacetamide (TAA). The spontaneous release of exogenous labeled taurine from superfused tissue slices was not affected in any brain area studied but the potassium-stimulated release was significantly enhanced in the striatum. An exposure of striatal slices to ammonium ions localized to the stratum pyramidale of CA1, systemically administered TMT effects are localized to cells bordering on CA1 striatum. At higher doses the selective effect was lost and cells with optimum pyramidal neurons were recruited by TMT and cells in the orienir cells are recruited by CA1.

While the effect of TMT on CA1 stratum pyramidale has been described at length previously, this is the first discussion of the preferential vulnerability to TMT toxicity of cells residing on the orienir border of the CA1 pyramidal cell layer. Recruitment of medial to lateral gradient of the selectivity of the TMT effect in CA1 are discussed as well as morphological aspects of the antiganglioside cells in CA1. The differences in selectivity between the two toxins are discussed with respect to possible mechanisms of TMT and KA toxicity toward Hippocampal CA1 neurons.

454.6


This study is to determine the in vivo effects of calcium ions from animal ca
cules, that results from exposure to specific fields, inhibit neuronal activity in vivo. Long term potentiation (LTP) and memory in the rat, when performing in a radial arm maze (RAME), have been shown to be causally dependent on movement of free calcium ions in animal cortex. Both effects rely on glutamate binding to the NMDA receptor which in turn causes conformational changes in the Ca2+ ion channel. The effects and the resulting Ca2+ currents are necessary conditions for LTP and RAM memory in rats. We are assessing RAM performance in rats while they are exposed to ELF and DC magnetic fields (MF). The exposure system produces a uniform field oriented horizontally within the RAM. The field strengths are 2 M of 2 x 10^-4 T (0.26 G) in combination with a 60-Hz MF of 5 x 10^{-4} T (0.5 G). Briefly, the RAM consists of eight equal length arms radiating out from a central area with a door at the entrance and a food cup at the end of each arm. Twenty-four male Sprague Dawley rats were food deprived to 80%-85% of their free feeding weight and then individually assessed in the RAM daily. Twelve rats were exposed during the RAM assessments, while the other 12 rats were sham-exposed. Presently the study is ongoing, but preliminary analysis of errors/group using a repeated measures ANOVA approach significance (p<0.05), with the exposed group making more errors. This data, when combined with results of a similar previous study, indicate a significant increase in errors made by the exposed groups (p<0.01). Work supported by DOE/ESD under Contract DE-AC06-76RL01830.

Cycasin and its aglycone methylezymeaminyl (MAM) are under study as etiological candidates of a prototypical human neurodegenerative disorder with taurine inclusion body formation in the lateral sclerosis, Parkinson's disease, and Alzheimer's disease. Cycasin was isolated from frozen Cycas circinalis L. seed kernels (Guam) and purified using UHPLC (Phenomenex Luna C18 column, 100% MeOH) to give crude cycasin, with levels of MAM (m%) that were determined by HPLC in media cultures treated for 5 days. Thin sections of explants were examined by light and electron microscopy.

At day 1, some cell bodies demonstrated vacuolated cytoplasm by light microscopy. By day 3, neuronal cell body necrosis was prominent and extensive vacuolization was observed in the neuropil. Vacuoles were marked increased in size at 5 days. Ultrastructurally, these vacuoles consisted of swollen dendritic processes, sometimes associated with recognizable synaptic terminals. Large vacuoles appeared to form from fusion of neighboring swollen processes. Nerve cell bodies also showed marked abnormalities, including patches of dense nuclear chromatin, aggregated ribosomes, swollen mitochondria, and a vacuolated cytoplasm. Stacks of membrane, which appeared to represent disorganized rough endoplasmic reticulum, were found in some tissues.

These observations provide direct evidence that crude cycasin, either directly or via a metabolite, has neurotoxic potential. Based upon the pattern of neuronal damage, the responsible agent might elicit neuronal damage via an excitotoxic mechanism. [Supported by NIH 19611]

455.3 PROCESSED CYCAD FLOUR EXTRACT NEUROTOXICITY IN NEUROLOGIC MURINE CELL CULTURE. M.C. D'Abreo, A.M. Marini, S.P. Kirby* and J.J. Kostick. CNB, NINDS, NIH, Bethesda, MD 20814.

A link between cycads and a variety of amyotrophic lateral sclerosis (ALS) in the western Pacific has been suggested. Therefore, we have tested extracts of female gametophyte tissue from C. circinalis, C. revoluta, and C. media, as well as cycas flour, for neurotoxicity using cultured cortical neuron cells and cultured brain cell cultures. Neither seed extracts nor 13 of the 17 processed flour samples were significantly more toxic than wheat flour. However, of 17 processed cycad flour extracts, one inhibited marked neuronal growth. Analysis of the extracts for the neurotoxin 2-amino-3-(methylamino)-L-propanoic acid (BMAA) indicated that there was no correlation between toxicity and BMAA content. The BMAA concentration of the medium was far below those required to kill cultured neurons. In addition, MK-801 did not protect against the neurotoxicity, indicating this response was not mediated via the N-methyl-D-aspartate (NMDA) receptor. The toxic principle could not be extracted into an organic solvent at acidic, basic or neutral pH and was heat and acid stable. All 4 toxic samples were subsequently found to have high content of zinc and this metal was shown to be responsible for the neurotoxicity. We conclude that cycad extracts are not significantly more toxic to cultured neurons than is wheat flour, and that the marked neurotoxicity of 4/17 processed cycad flour samples derived from C. circinalis was mediated via zinc. These findings link zinc to ALS on Guam, perhaps by exaggerating a long-term imbalance in essential trace minerals, or by the direct neurotoxic actions of zinc itself.


Soman, the toxic chlorohydride (CHF) inhibitor, causes chronic brain lesions through a yet unknown mechanism. The present study was designed to clarify the mechanism leading to soman induced brain lesions by comparing them to the effect of either a soman convulsant (metrazol) or another toxic CHF inhibitor (DFP) in equivalent (LD50) doses. Following administration, surviving rats were sacrificed at various time intervals and their brains evaluated by histological and morphometric analysis. All three substances produced convulsions, including convulsions. Soman and DFP also generated signs of chronic brain lesions (injection). Soman injected rats developed CNS lesions already 24 hr after administration, mainly in the hippocampus, frontal and parietal cortex and the hypothalamus. In these lesions these areas had spread to areas which were not involved initially. Morphometric analysis revealed that the dynamic pattern of lesion morphology, as well as the pattern of lesion morphology, was changed in any of the exposed brain areas, not by convulsions per se but by CHF inhibition per se.

455.6 EFFECTS OF THREE REPUTED CARBOXYLASE INHIBITORS UPON RAT SERUM ESTERASE ACTIVITY. J.P. Chambon*, S.L. Hartgroves*, M.R. Murphy* and L.J. Valdes*. The University of Texas at San Antonio, San Antonio, TX 78229; The University of Texas, San Antonio, TX 78235 and U.S.A. Army Chemical Research, Development and Engineering Center, Biotechnology Division, Aberdeen Proving Ground, MD 21010.

Rats have very high androgenous levels of serum carboxylesterase (CAE) which accounts for the lower sensitivity of rats to toxic organophosphates. The effects of three reputed CAE inhibitors, 2-0-Cresyl-4H-1:3:2-benzodioxaphosphorin-2-oxide (CBP), bis-p-nitrophenyl-phosphate (BNPP) and tetraisopropyl pyrophosphate (ISP-ONPA) on the metabolism of several substrates were determined. These kinetic constants, apparent Vmax were derived and inhibitory effects compared using saturating amounts of substrate. Using lab polyacrylamide gel electrophoresis and densitometric scanning, the effects of these inhibitors upon hydrolysis of various substrates by rat serum esterases were monitored and quantitated. Research supported by APOGR.
455.7 THE USE OF CARBOXYLESTERASE INHIBITORS TO DEVELOP AN IMPROVED BOVY MODAL OF SOMAN TOXICITY. M.R. Murphy, S.J. Kerényi, S.A. Miller, J.P. Chambers, R.F. Monaghan, and S.L. Hartgraves. USAF School of Aerospace Medicine, Brooks AFB, TX 78235.

Carbohydrolases (CAs) (aka esterases) are non-specific C-reactive enzymes of unknown function, except possibly the detoxification of xenobiotics. Because their catalytic sites are harbored by a large number of species, this species is much less sensitive, relative to primates, to highly toxic organophosphate anticholinesterases such as soman. The purpose of our work was to develop the CAE-inhibited rat as an improved (i.e., more primate like) model of soman toxicity.

We report the results of studies on (1) the more frequent re-exposure of the CAE inhibitors (CAs) to the biological and non-specific anticholinesterases (i.e., measured by the reduction in brain cholinesterase (Che), serum Che, and spontaneous activity; (2) the dosages, in brain cholinesterase (Che), serum Che, and spontaneous activity; (3) the 7-day chronic inhibition of CAE, and (4) the effects of CAEs on cholinesterase (Che), serum Che, and spontaneous activity. Research was supported by the USMRRCD.


RADCCTI-I, U.S. Air Force School of Aerospace Medicine, Brooks AFB, TX 78235. UTSAA.

STS is a soman-induced disorder characterized by severe, localized, brain damage and hyperreactivity. This can be shown in the studies of Smith et al. (1986). The purpose of this study was to investigate the nature of this syndrome and to determine the potential for development of a control group (SIG). The weight loss control (WLC) group was included to approximate the severe weight loss and subsequent recovery of the STS group. Animals developing STS received extra care (e.g., special diet, dextrose injections, and bedding), and WLC animals were treated similarly.

STS animals displayed hyperreactivity to stimuli in a two-way shuttlebox, but learning was unaffected. However, STS animals did exhibit a loss of long-term potentiation in the ipppp hippocampus. In automated activity monitors, STS rats habituated rapidly to the novel environment, and exhibited a total exploratory behavior. When given 1.5 mg/kg of amphetamine, STS animals showed a much more dramatic increase in activity than did WLC animals (Hartgraves et al., these proceedings).

455.9 EFFECTS OF STIMULANTS ON LOCOMOTOR ACTIVITY IN RATS WITH SOMAN TOXIC SYNDROME (STS). S.L. Hartgraves, M.S. Triantafyllopoulos, S. Z. Kerényi, S.A. Miller, and R. Murphy. USAF School of Aerospace Medicine, Brooks AFB, Texas, 78235-5301; University of Texas Health Science Center, San Antonio, Texas, 78284.

Soman Toxic Syndrome (STS) is a soman-induced, seizure-related syndrome characterized by extensive, but localized, brain damage, hyperactivity to physical stimuli, and general hypoactivity (see abstract by S.A. Miller et al., this conference). The objective of this research is to further analyze the locomotor behavior (measured by automated activity monitors) of STS animals by administering stimulant drugs.

STS animals respond to d-amphetamine (1.5 mg/kg, i.p., a.c.) with 3-fold increases in distance traveled and rearing compared to weight loss controls (WLC). STS animals also show over 20-fold increases in these same parameters compared to their responses to saline injections, whereas WLC animals show only 3-fold increases in distance traveled and rearing (d-amph vs. saline, crossover).

We report here a comparison of STS dose-response curves for d-amph and for caffeine, a stimulant that enhances locomotor activity independently of the nucleus accumbens (which is closely associated with d-amph-induced locomotor activity).


Acetylcholinesterase (AChE) in the clonal NG108-15 cell line has been previously characterized. This cell line may represent a model system in which to study ACHE regulation and effects of chemical compounds that may alter ACHE activity (Ray et al. Soc. Neurosci. Abstr., 12:899, 1986). It has been shown that glycol-l-glutamine (GLG) may function as a neurotrophic factor for maintenance of ACHE content in denervated superior cervical ganglion of the cat (Koelle et al., 1988). Studies were conducted in undifferentiated NG108-15 cells to characterize the regeneration of ACHE activity after soman inhibition. In addition, the effect of GLG on ACHE activity after soman treatment was investigated. Cells were grown to confluence, treated with soman (10^-8 M) for 15 min and washed to remove excess soman. GLG (10^-5, 10^-4 or 10^-3 M), glycol-l-glutamic acid (Gly/LG) or a combination of the two was added to the culture medium immediately after soman treatment and maintained in the medium until cell harvest. Cells were harvested at 1, 3, 5, 12, 20, and 24 hours after soman treatment and specific ACHE activity was determined. After soman, ACHE activity dramatically decreased to less than 1% of control activity at 1 hr. The regeneration of ACHE activity gradually increased at 5% (7%), 12% (14%) and 20 (25%) hours, while complete ACHE activity was regained 24 hr after soman. GLG and glycol-l-glutamic acid, at all concentrations tested, did not increase the rate of ACHE in apo synthesis after soman inhibition. These studies have characterized ACHE regeneration after soman inhibition in the NG108-15 cell line for potential use as a model system to study compounds that alter ACHE activity. In this model system, GLG and glycol-1-glutamic acid failed to act as a neurotrophic factor for ACHE synthesis.

455.11 HYPERAMMONEMIA, IN THE ABSENCE OF GLUTAMINE SYNTHESIS, DOES NOT ALTER BRAIN FUNCTION. J. Dessau*, M. DeLegge & A. Hawkin. Dept. of Physiology and Biophysics, University of Health Sciences / The Chicago Medical School, North Chicago, IL 60064.

Portacaval shunted rats show many of the typical metabolic alterations found in humans with hepatic encephalopathy. Most of the characteristic changes are established within 24-48 h after surgery and include: raised plasma and brain ammonia, depressed brain function and increased transport of neutral amino acids across the blood-brain barrier. Ammonia is suspected to be an important etiologic factor. An earlier study from this laboratory showed that hyperammonemia caused most of the changes found after portacaval shunting and that the depression of cerebral function was more closely related to glutamine, a metabolite of ammonia, rather than ammonia itself. The present experiments were designed to address the question: what happens to ammonia alone, in the absence of net glutamine synthesis in brain, could be responsible for cerebral dysfunction. Plasma ammonia levels in normal rats were raised to values similar to those found after portacaval shunting by the administration of small doses of methionine sulfoximine, an inhibitor of glutamine synthetase. Cerebral energy metabolism in methionine sulfoximine-treated rats remained unaltered. There was no depression in brain glucose consumption. Key intermediary metabolites and high energy phosphates were normal and neutral amino acid transport (tryptophan and leucine) was unchanged. These results indicate that hyperammonemia, when not accompanied by net glutamine synthesis in the astrocytes, does not result in cerebral dysfunction. (Supported by NSF Grant 26389 and NS 16737).


The brain arylamine N-acetyltransferase (NAT) activity is about 10-fold higher than the arylamine NAT present in the same tissue. The function and regulation of the arylamine NAT is not known at this time. Recent molecular cloning studies indicate that multiple arylamine NATs, based on their substrate specificity, may exist in different tissues. In the present study, we have investigated the above possibility with respect to rat brain.

Arylamine NAT activity was assayed in microscale samples, using p-phenoxydine as the amine substrate, and was found to be evenly distributed in different areas of the brain. The enzyme activity was purified via a three step procedure involving (N15)5H04, precipitation, affinity chromatography using methionine, and finally, size exclusion HPLC. Analysis of the final purification using SDS-PAGE showed one major band (Mr=36K, 375K) and a few minor bands. The Mr of the native enzyme was found to be approximately 101K. Substrate specificity studies indicate that the enzymes are arylamine NAT, with only 1% activity towards arylalkylamines (i.e. tryptamine). The remarkably high specificity of this enzyme towards arylamine NAT, enzyme may differ from that found in liver. (Supported by NIH grant, DK 37024 to MAAN).

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
455.13

Chronic ingestion of the nitroarylphatic neurotoxic 3NPA, a suicide inhibitor of SDH, results in intraneuronal glycerogen accumulation in the spinal cord in young mice. The acute single dose LD50 was identical in young [221 mg/kg; 95% confidence limits: 218-222 mg/kg] and old [49 mg/kg; 47-51] mice. Following daily gavage feedings, the chronic LD50 was significantly [p<0.04] higher in old [118 mg/kg; 114] compared with young [49 mg/kg; 47-51] mice. Sucinonic dehydrogenase [SDH] activity was decreased at 10 min but was absent at 30 min post 3NPA. Spinal cord intraneuronal glycerogen accumulation was not seen in mice acutely fed 2.5 LD50 but was present following chronic feeding of 0.2 mg/kg 3NPA. Aged mice demonstrated no increased resistance to acute toxic effects of 3NPA on spinal cord neurons but could withstand a 3-fold increase in the dose of chronically administered 3NPA. (Supported by NASA Midwest Regional ALS Research and Treatment Program grant and an ADA Research Fellowship grant).

455.14
SEX-RELATED DIFFERENCES IN TRIMETHYLPOLYPHOSPHATE NEUROTOXICITY AND PHARMACOKINETICS, J. Ross, III, Y. J. Fornatt and J. Gearhart, Naval Medical Research Institute, Toxicology Detachment, Wright-Patterson Air Force Base, Ohio 45433 and NII Technology Services Corporation, Dayton, Ohio 45431.

Thermal decomposition of trimethylpolyporphosphate-based compounds which contain fire retardant trivalent phosphorus additives allows formation of toxic trimethylpolyporphosphate (TMP-P), a caged convulsant believed to act via GABAergic receptor antagonism.

The effect of sex-related differences after dermal applied TMP-P was evaluated in rats (Fish 344) with hormonally-altered sex phenotypes and in castrated adult rats with and without hormonal supplementation. Auditory startle was found to be greater in males than females after a subcutaneous dose of TMP-P (30 mg/kg). Additionally, males exhibited shorter times to convolution and death than females after a lethal dose (120 mg/kg). Female rats dermally exposed to TMP-P exhibited greater tissue/blood partition coefficients than males. Phenotypically and surgically unaltered animals exposed to TMP-P or the solvent vehicle served as the positive and negative controls, respectively. A physiologically based pharmacokinetic (PBPK) model was developed to describe the kinetics of TMP-P. The steady state partition coefficients of TMP-P in tissues and urinary excretion rates, determined in subcutaneously dosed animals, was used in the PBPK model to estimate the in vivo metabolism of TMP-P and to simulate the blood and tissue kinetics. The PBPK model was then used to derive a permeability rate constant for TMP-P transfer across rat skin and to explain the effects of tissue/blood partitions on brain levels of TMP-P.

455.15

The use of streptozotocin in animal models has shown to develop abnormalities in the nerve similar to those observed in diabetes. Reduced Na-K ATPase activity is often reported for the peripheral nervous system. This study established the Na-K ATPase in the regions of the brain during diabetes. Streptozotocin (60mg/kg, i.p.) was injected i.p. in an epidural fashion at 21/2, with battery packs buried subcutaneously at the incision. Stimulator intensities ranged from 0 to 18 uA. The animals were allowed to survive for a period of 2-12 weeks undergoing twice weekly inclined plane testing. Following this, the animals were sacrificed and the hindlimbs were harvested and sectioned at 8 uM, and stained with Luxol fast blue and Hemotoxylin and Eosin. Grossly, pathologically changes such as discoloration and cavitation were seen under the anode after 3 weeks, with intensities as low as 3 uA. Inclined plane testing was sensitive only to the 18 uA induced changes. These results demonstrate the low tolerance of mammalian CNS tissue to DC stimulation and have implications for continued studies into its therapeutic usefulness.

455.16
NEUROTOXICITY FROM DIRECT CURRENT STIMULATION, R.J. Hurlich, E. Theriault, C.H. Tator, Playfair Neuroscience Unit, University of Toronto, 399 Bathurst St., Toronto, Canada M5T 2S8.

The effect of continuous direct current stimulation on the normal adult rat spinal cord was assessed for a variety of intensities in 32 animals. Platinum/iridium stimulating electrodes of 2 sq.mm. surface area were implanted 10 mm. anterior to the obex. Stimulator intensities ranged from 0 to 18 uA. The animals were allowed to survive for a period of 2-12 weeks undergoing twice weekly inclined plane testing. Following this, the spinal cords were harvested and sectioned at 8 uM, and stained with Luxol Fast Blue and Hematoxylin and Eosin. Grossly, pathologically changes such as discoloration and cavitation were seen under the anode after 3 weeks, with intensities as low as 3 uA. Inclined plane testing was sensitive only to the 18 uA induced changes. These results demonstrate the low tolerance of mammalian CNS tissue to DC stimulation and have implications for continued studies into its therapeutic usefulness.

455.17
NEUTRON/GAMMA IRRADIATION PRODUCES DIFFERENTIAL LOCOMOTOR DECREMENTS IN ISOLATED AND GROUP HOUSED MICE, H.D. Davis, M. Mirnichenko, M.E. Faccioni, and M.B. Landau, Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5165.

Males CDF1 mice that were either isolated for 5 days or group housed (10/cage) to 5 Gy of radiation at 1 Gy/min (neutrons/gamma = 1:1), or sham irradiated (control) (N=16/group). Locomotor activity (ambulation) of individual mice was monitored for 12 hr postirradiation (PR). There was no significant difference in activity between the isolated and the group housed controls. Irradiated mice exhibited a locomotor decrement within 1 hr PR that fell to control levels by 6 hr PR in the group housed animals and 62% of control levels by 3 hr PR in the isolated animals. Those administered intraperitoneal saline alone were not significantly different from the control group. However, irradiation-induced locomotor decrements in isolated animals were not significantly different from the isolated control animals by 4 hr PR while the group housed irradiated animals remained significantly different from their controls until 8 hr PR. Thus, irradiation-induced locomotor decrements in isolated and group housed controls exhibited similar levels of activity. These findings represent differential effects of radiation in animals housed under different social conditions, with social isolation attenuating the behavioral effects of radiation.

455.18
MODIFICATION OF ACOUSTIC STARTLE REFLEX FOLLOWING EXPOSURE TO IONIZING RADIATION, D.E. Morse and K.M. Manderfield, Behav. Sci., AFRL, Bethedha, MD 20814.

Inhibition of the acoustic startle reflex, by the presentation of a pre-startle pulse stimulus, has been shown to be sensitive to changes in sensory and motor capacity following total body exposure. It has been reported that exposure to ionizing radiation, at sublethal doses, severely depletes locomotion and appetitive behavior. The data suggest that these behavioral effects are related to changes in endogenous opioid and dopamine activity. It is not clear whether changes in locomotor capacity or sensory function contribute to these effects. In the present study, male Sprague Dawley rats (200-345g) were exposed to ionizing radiation (1-10 Gy, Linac 18 MVP electrons, 10 Gy/min), or were sham irradiated. Following each dose, the startle response (Startle pulse: 122 dB, 25 ms dur., 2 ms rise), on trials where a pre-stimulus preceded (100 ms) the startle pulse, was compared to startle amplitude of the startle stimulus (Startle pulse: 122 dB, 25 ms dur., 2 ms rise), on trials where a pre-stimulus preceded (100 ms) the startle stimulus was presented alone. Baseline responding was measured in a separate session. The results suggest that neither radiation exposure or opioid blockade affected the amplitude of the startle stimulus or the startle-elliciting stimulus (when presented alone). However, prepulse inhibition of the startle reflex was significantly reduced following radiation exposure. The data suggest that postirradiation behavioral suppression is not caused by decreased motor capacity, but instead may be mediated by changes in sensory function.

Protein kinase C (PKC) is an important enzyme in mediating cellular signal transduction. Recently it has been reported that subcellular concentrations of lead activate partially purified PKC from rat brain (Nature 334, 71-73, 1988). Since lead is a neurotoxic heavy metal and its toxicity may be related to the alteration of PKC activity in vivo, we examined lead effects on PKC activation. PKC from brains of Sprague-Dawley rats was purified using a zymosan-Sepharose column followed by affinity chromatography using peptide substrates I, II and III were further separated using a hydroxyapatite FPLC column.

Our results showed that lead had strong inhibitory effects on PKC activity of these subtypes as follows:

1. Submicromolar concentrations of lead acetate strongly inhibited PKC activity induced by phosphatidylserine (PS) in the presence of calcium. Lead did not mimick the calcium effect on PKC activation in the presence of PS.
2. Dithiophosphate (DTPA) activated PKC without calcium. This activation of type I and II PKC was also inhibited by lead acetate at the same concentrations.

Such strong inhibitory effects of lead on PKC may be related to its neurotoxicity, such as memory disorders.
545.3 COMPARISON OF METHODS FOR THE HIGH THROUGHPUT (96 WELLS PLATE) DETERMINATION OF LDH RELEASE FOR ASSESSMENT OF NEUROTOXICITY IN LOW DENSITY NEURONAL CULTURES. D. L. Needels, CNS Biology, Brussels, Munich, Munich.

Neurotoxicity can be assessed in vivo by determining 545.4 CHOLINERGIC SEPTOHIPPOCAMPAL NEURONS UNDERGO MORPHOLOGICAL ABERRATIONS FOLLOWING LOCAL APPLICATION OF THE NEUROFILAMENT-DISRUPTING AGENT 2,5-HEXANEIDONE (HD). P. L. Di Patro and L. L. Butchak. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563, U.S.A.

We previously demonstrated that intrahippocampal (i.h.) injection of the microtubule-disrupting agent colchicine induces morphological and functional alterations in fibers running through the distal limbia and stained with acetylcholinesterase (AChE) and nerve growth factor receptor (NGFR) (Di Patro et al. Brain Res., 1995, in press). We investigated the developmental effects of i.h. injection of HD, a drug known to produce axonopathies characterized by local accumulations of neurofilaments. Adult Sprague-Dawley rats were used and i.h. stereotaxic injections were carried out as previously reported (above). Two and 4 days after i.h. injections of HD 0.5 mg, we found that many AChE-NGFR positive axons in the most rostral part of the limbia were more thick and grossly distorted as compared to fibers in the homologous region of control rats. At 7 days post-treatment, the affected fibers became more distorted and showed occasional thin collateral branches, which resembled newly sprouting ramifications.

Reduced number of choline acetyltransferase-reactive neuronal bodies in the lesioned hemisphere was also observed. A complete recovery of all these changes was seen 6 weeks after HD-treatment. Our data show that i.h. injections of cytoskeletal toxins may constitute a useful experimental model to study the role of the different cytoskeletal components in controlling axonal shape and in regulating the growth of fibers. [Support: NS 10928]

545.5 INCREASE IN CORTICAL BRAIN MICROVASCULARIZATION FOLLOWING DRUG ADMINISTRATION, OPTICAL INSURANCE, AND IN THE RAT. M. N. Gemis*, Ch. Heidbruder* and Ph. De Witte, Lab. of Psychology, University of Louvain, Croix du Sud 1, B-1340 Louvain-la-Neuve, Belgium.

The alcoholization by an inhalation procedure seems to be one of the most appropriate experimental models to obtain and maintain chronically high blood ethanol levels. After subcutaneously 2 to 4 weeks into the alcoholization chamber, rats were perfused with a nuclear emulsion allowing to reveal the cerebral vascularization. Furthermore, we also examined the issue of whether chronological ageing and cortical lesion were able to modify cortical microvascularization in vivo. In non-alcoholized rats, the vessels including terminal and lateral branches, were measured and the lengths were summed up. Our results provide evidence that a small brain was less alcoholized from 2 to 4 weeks displayed enhanced cortical microvascular network. A similar enlargement of microvascularization was observed in the cortex of aged animal compared to normal adult rats and at the edge around a lesion-induced cavity performed in the cortex of non-alcoholized rats by contrast to the contralesional intact side on the same section. These results show that chronic alcoholization, chronological ageing and brain injury share in common cortical hypervascularization.

545.6 MODULATION OF \textsuperscript{35}S-BUTYL-BICYCLOPHOSPHOROXYHATONE KINETICS BY DELTAMETHRIN IN RAINBOW TROUT BRAIN MEMBRANES. R.L. Golden and T.F. Murray. Toxicology Program and College of Pharmacy, University of Florida, Gainesville, Florida.

Pyrethroid insecticides are known to attenuate \textsuperscript{35}S-buty1-bicyclophosphorylhydantoin binding to gamma-aminobutyric acid (GABA) gated chloride channels in the central nervous system. Deltamethrin, a pyrethroid, has been shown to inhibit the equilibrium binding of \textsuperscript{35}S-STIRPS in rainbow trout membrane preparations (Edelman and Murray, Neuropharmacology, in press). To investigate the mechanism of deltamethrin inhibition of \textsuperscript{35}S-STIRPS binding we studied the influence of deltamethrin on the kinetic of \textsuperscript{35}S-STIRPS binding to trout brain membranes. Deltamethrin modified \textsuperscript{35}S-STIRPS association and dissociation were both adequately described by a single component model. In contrast, in the presence of GABA both association and dissociation of \textsuperscript{35}S-STIRPS binding were better characterized by a two component model. In the presence of GABA the \textsuperscript{35}S-STIRPS association rate increases as a function of GABA concentration. Deltamethrin was found to affect \textsuperscript{35}S-STIRPS association in a GABA dependent manner. Deltamethrin effected a reduction in the rate constants for both the fast and slow components of association. In contrast, deltamethrin had no effect on the \textsuperscript{35}S-STIRPS dissociation rates. These results suggest that the mechanism of the apparent inhibition of equilibrium binding of \textsuperscript{35}S-STIRPS by deltamethrin is a reduction in the association rate of this reaction. (Supported by NIH Grant ES0499/)


Direct administration of polychlorinated biphenyl (PCB) to adult animals, indirect provision to rat pups via the maternal diet, results in depression of cholinergic status. Concurrent with hypercholineraemia, PCB (25 ppb) decreases basal activity of choline acetyltransferase (ChAT) in hippocampus and basal forebrain at 15 days of age. In the present study was done to determine whether injection of choline homone (Chromax, 5 or 500 micrograms/500 

545.8 A WINDOW OF VULNERABILITY FOR DEVELOPING DOPAMINE RECEPTORS? M.L. Schmidt* and R. L. Schm. Psychopharmacology Unit, Clin. Inst. of Psychiatry, Toronto, Canada, MST 1B6, and Dept. of Pharmacology, Univ. of Toronto, Toronto, Canada, MST 1AB.

Prolonged prenatal blockade may impair ontogenic dopamine receptor acquisition in rat striatum (Rosenkranz and Friedhoff, Science, 201: 1133, 1979; Scalzo et al., Pharmacol. Biochem. Behav., 35: 721, 1989). A window of vulnerability from embryonic day 15-18 has been described (Rosenkranz et al., Birth Devel. 19: 511, 1983), but has not received recent attention.

To test the hypothesis that blockade during this brief period is sufficient to impair dopamine D2 receptor acquisition, pregnant Wistar rats were given haloperidol, thiothixene or trifluperazine at doses of 2.5, 5.0 or 7.5 mg/kg/day s.c. from gestational day 15-18. Pups were sacrificed 14 days after birth. Density and affinity of dopamine D2 receptors in pooled striatal membranes of each litter were determined by Scatchard analysis, using \textsuperscript{3}H)-spiperone as a ligand. In control tissues, density was found to be 110 ± 2 fmol/mg, while affinity was 0.18 ± 0.03 nM (x ± s.e.m.). The three drugs did not produce consistent, dose-dependent changes in D2 receptor density or affinity.

The results of this study suggest that the brief period of exposure is insufficient to impair D2 receptor ontogeny and call into question the concept of a window of vulnerability. [Supported by the Clarke Institute Research Fund and Ontario Graduate Scholarship Program (M.S.S.)]

A variety of unique compounds has been obtained as the result of the investigation of the metabolism of 5-hydroxytryptamine (5-HT) and related indolamine species. Some of these compounds have exhibited substantial toxic effects when administered intracerebrally in mice. Some have led to interesting behavioral reactions following such administration. In order to establish an appropriate dose at which to examine the effects of these species on endogenous neurochemical levels, preliminary investigations examined the LD50 values for each of the Dixon-Upjohn method. (W.J. Dixon, J. Am. Stat. Assoc., 46, 967, 1965). The results obtained were:

<table>
<thead>
<tr>
<th>Compound</th>
<th>LD50 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-hydroxytryptamine-4,7-dione</td>
<td>&gt;150</td>
</tr>
<tr>
<td>5,7-dihydroxytryptamine-4,5-dione</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>6,6'-bi-(5-hydroxytryptamine-4,7-dione)</td>
<td>52 ± 1</td>
</tr>
<tr>
<td>5,6,7-trihydroxytryptamine</td>
<td>&gt;150</td>
</tr>
<tr>
<td>2,7,8,9,10-pentahydroxy-tryptamine</td>
<td>&gt;150</td>
</tr>
</tbody>
</table>

Endogenous levels of catecholamines, indoleamines, and acetylcholine, as well as related metabolites, were determined using a NEUBA* Neurochemical Analyzer. This system provides valuable qualitative information in the identification of the pertinent species through the use of multiple amperometric electrochemical detectors. It provides rapid throughput by using a multichannel liquid chromatographic setup. Behavioral effects observed for the compounds listed above included hyper-excitability, sedation, short-term (72 hr) partial motor impairment, and rapid "rolling over."
461.15
MOLECULAR CLONING AND ANALYSIS OF mRNA EXPRESSED IN TRIMETHYL-TIN-SENSITIVE NEURONS. S. M. Togus*, J. K. Knoll*, J. W. Polli* and M. L. Billingsley. Department of Pharmacology and Center for Cell and Molecular Biology, Penn State University College of Medicine, Hershey, PA 17033.

Trimethyltin (TMT) is a selective neurotoxicant that destroys neuronal populations which have no apparent neurochemical or anatomic relationships. To isolate gene products common to sensitive neurons, a cDNA subtraction library was created using avidin/biotin-based methods. One clone, pr9T19, gave patterns of hybridization in Northern and in situ hybridization experiments suggesting that it was expressed in TMT-sensitive neurons. Strong hybridization was seen in cingulate and piriform cortex and hippocampus. pr9T19 was expressed in rat telencephalon on embryonic day 15; the pattern of expression became restricted to the adult pattern by postnatal day 20. In situ hybridization in human hippocampus suggested that an mRNA related to pr9T19 was expressed in subpopulations of hippocampal pyramidal cells. High stringency Southern blot analysis indicated that a mixed gene was present in drosophila, rabbit, rat, and human genomic DNA. Initial sequence analysis suggests that the 3.0 kb mRNA encodes a novel protein of 123 amino acids. Experiments are underway to characterize this protein and to determine its possible role in the neurotoxicology of TMT.

DEGENERATIVE DISEASE—OTHER: BASAL GANGLIA

467.1
PROGRESSION OF BASAL GANGLIA ATROPHY AND FUNCTIONAL DECLINE IN HUNTINGTON'S DISEASE PATIENTS. J.A.A. Roberts and R.A. Loeh, Dept. of Neuropsychiatry, Royal Ottawa Hospital, Ottawa, Canada.

Neuroanatomical and functional changes associated with the progression of Huntington's disease (HD) were investigated. CT scans were obtained from 14 individuals at various stages of HD. Scans were digitized, measures of (1) distance between frontal horns of lateral ventricles (FH), (2) distance between caudate nuclei (BICAUD), and (3) width of globus pallidus and putamen regions (GP) were calculated. These measures were correlated with patient's scores on the Quantitative Neurological Examination (QNE), Shoulson and Fahn's functional assessment-Motor, and Mini-Mental State (MMS) scales. Measures of the duration of HD and the GP and BICAUD regions were significantly correlated (r = .70). Further, the GP measure was again correlated with MMS and (r = .90) and S&F (r = .81). Scores more specific to the caudate regions: FH and BICAUD were more strongly related to the motor function score QNE (r > .85). This indicates that atrophy of the GP region may be a better index of functional deterioration than the more commonly used measures of caudate atrophy which were more closely related to motoric decline.

467.3
PATHOLOGICAL CHANGES IN EARLY HUNTINGTON'S DISEASE. J.C. Hedreen. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The distribution of histopathological changes in autopsied cases of Huntington's disease (HD) with different pathological grades of severity was examined by means of gial fibrillary acidic protein immunocytochemistry and counts of neurons and astrocytes. Two principal findings were made. First, in three presumed grade-0 HD cases, it was determined that neuronal counts in the dorsal putamen were less than counts in controls; this finding provides a significant aid to diagnosis in very mildly affected cases. Second, in many grade-2 and grade-3 cases, a nonuniform pattern of pathology was seen at the ventrally advancing margin of pathological change. In this region, areas with neuronal cell loss and gliosis were interspersed with relatively normal areas, forming patterns reminiscent of those created by the intrinsic chemarchitecture of the neostriatum and by striatal afferents. This finding suggests that neuronal injury in HD may initially involve neurochemically specific cell groups in the striatum or striatal zones innervated by specific afferent systems. Identification of these specific cell groups or afferents will have important implications for understanding the mechanism of neuronal injury in HD.

467.4

Numbers of neurons at up to three strictly defined anatomical levels of the locus coeruleus (LC) were determined in 33 patients with Huntington's disease (HD) and in 22 age-matched controls. Distances between rostral and caudal levels (LC length) could be determined in 23 of the HD and in 7 of the control cases. Rostral level cell counts were significantly less in HD patients who had severe dementia than in controls and, at rostral and middle levels, and HD patients with severe dementia compared to those with mild dementia. Fewer rostral and middle level LC neurons correlated with neuropathological and last recorded motor impairment (MIS) and activities of daily living (ADL) scores. LC length was reduced by >20% in patients with severe dementia compared to those with mild dementia (p < .002) or controls. Reduced LC length correlated with early age of onset, long duration, MIS, and most highly, ADL score (Pearson r = -.72; p < .001). In conclusion, LC pathology, especially as reflected in LC length, correlates highly with clinical features of advanced disease.
DEGENERATIVE—OTHER: BASAL GANGLIA
THURSDAY

457.7
MET-ENKEPHALIN, SUBSTANCE P, AND GABA LEVELS IN RIGID AND CHOREIC HUNTINGTON’S DISEASE PATIENTS.
E. Storey and M.F. Beal.

It has recently been suggested on the basis of immunohistochemical studies that choline in Huntington’s disease (HD) results from early depletion of net-enkephalin (ME)-containing and GABAergic neurons in the external segment of the pallidum (GPi), with initial relative sparing of substance-P (SP)-containing GABAergic neurons projecting to the internal segment of the pallidum (GPI). The imbalance between these opposing pathways is thought to result in disinhibition of the ventrolateral thalamus, and increased excitatory input to the cortex, resulting in chorea. In an attempt to confirm this hypothesis, brains from 9 adult chonic HD patients were compared with 9 adult and 6 juvenile-onset rigid cases, and with 12 control brains. All patients were examined clinically within 6 months of death. All but one of the affected brains were grade 3 or 4. There were significant differences in SP, ME, and GABA levels in grade 3 versus grade 4 cases. However, measurements of ME and GABA levels by RIA failed to show any preferential preservation of SP versus ME in choreic cases, and indeed ME was better preserved than SP in the striatum. Measurement of GABA levels by HPLC also failed to show preferential preservation in the GPI in cases with chorea versus those with rigidity. The findings were unchanged when only choreic (6) and rigid (5) grade 3 cases were compared. SP, ME, and GABA in the GPI were not significantly different from depletions of ME and GABA in the GPI in all cases were compared. There was, however, a trend towards relative preservation of GPI SP and GABA in chonic patients. Although a preferential loss of ME-containing GABAergic projection neurons to the GPI may play a role in the genesis of chorea, these results did not demonstrate this in advanced cases.

457.9
MUSCARINIC RECEPTORS IN BASAL GANGLIA OF PROGRESSIVE SUPERNUCLEAR PALSY (PSP). (P). N. S. Hermanowicz, J. B. Perry, N. L. Foster, I. Shoulson,
Dept. of Neurology, University of Michigan, Ann Arbor, MI 48104-1687 and University of Rochester, Rochester, NY 14642.

PSP is characterized by severe nigtal, subthalamic, superior collicular and pallidal atrophy with lesser striatal pathology. Choline acetyltransferase and dopamine D2 receptors are reported to be increased in subcortical areas (Kroger et al. Ann. Neurol. 18:523,1985). We have measured muscarinic cholinergic receptors using autoradiography in basal ganglia of 8 PSP brains and compared them to 11 control, 9 Huntington’s (HD) and 7 Parkinson’s (PD) disease brains.

Whole coronal, cystal sections through the medial globus pallidus were incubated at 20°C for 3 hrs. in 1 mCi [3H]QNB (Amersham) in 50 mCi Tris-HCl. Sections were exposed to Ultrascan-XL (K.B.) for 2 weeks and analyzed with an MCI (Imaging Research). Binding in pitting protein (mean±SEM).

<table>
<thead>
<tr>
<th>Disease</th>
<th>Control</th>
<th>PSP</th>
<th>HD</th>
<th>PD</th>
<th>PD (*</th>
<th>ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caerulein</td>
<td>2.9±0.2</td>
<td>2.1±0.1</td>
<td>2.5±0.6</td>
<td>2.5±0.6</td>
<td>2.5±0.6</td>
<td>2.5±0.6</td>
</tr>
<tr>
<td>Pentedate</td>
<td>2.06±1.1</td>
<td>1.45±0.1</td>
<td>2.56±0.3</td>
<td>3.38±1.7</td>
<td>2.56±0.3</td>
<td>3.38±1.7</td>
</tr>
<tr>
<td>PD</td>
<td>0.27±1.4</td>
<td>2.16±1.7</td>
<td>2.19±0.2</td>
<td>2.96±0.1</td>
<td>0.27±1.4</td>
<td>2.16±1.7</td>
</tr>
</tbody>
</table>
| (* p<0.05 by ANOVA with post hoc pairwise comparisons to control)

In HD striatal cell loss is much more severe than pallidal. Thus, the decreased pallidal QNB binding in HD suggests that these binding sites are on striatal efferent terminals. Decreased pentedate QNB binding in PD indicates a loss of cells bearing muscarinic receptors in putamen. The increased medial pallidal binding suggests a concentric, myelinated projection of glial-like periventricular QNB binding sites in PD may be on a spared subset of striatal efferent terminals. Supported by USPHS grant AG06711.

458.8

Spiny striatal projection neurons are most severely involved in Huntington’s disease (HD). A major subcortical target of these neurons is the globus pallidus (GP). Striatal terminal projections to GP have been identified with a variety of neurochemical markers. We stained continuous, 50-μm-thick sections of the GP in 17 neurologically normal control and 16 HD brains for substance P (SP) and met-enkephalin (ME) and compared findings of SP-activity in ME- and ME-positive neurons in HD. GP was stained with the electron dense reaction product of the Alzet pump (3H)/H3. GPe and SN were stained with [3H]3,4-dihydroxy-3-(3-hydroxykynurenine) (3-HKYN) and used as markers for SP-activity, as the injection of this tracer into the striatum. SP-activity in ME- and ME-positive neurons was significantly increased in the striatum of HD. SP-activity was present in the spongione zone of the L-KYN and SP-activity induced lesion. Quinolic acid, the terminal metabolite of L-KYN, was not neurotoxic. The neurotoxic potential of kynuramine is unknown, while the role of the neurotoxic quinoline in HD is doubtful. The presentation of a specific neurotoxin in specific HD families may reflect subtle errors within the HD gene locus. Supported by NIMH grants 1R01-NS22037-01A1.

459.10
ABNORMALITIES OF DOPAMINERGIC MARKERS IN POSTMORTEM STRIATAL SPECIMENS FROM PATIENTS WITH TOURETTE SYNDROME Harvey S. Singer, In-Hel Hahn, Kimberly H. Moran
The Johns Hopkins University School of Medicine.

Baltimore, Maryland 21205.

The dopamine hypothesis for Tourette Syndrome (TS) was evaluated in frozen postmortem striatal tissue from three adults (2 males, 1 female) with the diagnosis of TS and up to 13 controls. [3H]mazindol binding (fmlg/mg protein), used to label the DA uptake carrier site, was significantly increased in the striatal region of patients with TS (caudate: TS = 467 ± 22, control = 342 ± 28; putamen: TS = 604 ± 37, control = 404 ± 43). HPLC measurements of dopamine and its metabolite DOPAC were normal to slightly reduced. [3H]jCH339 binding, labels the D1 receptor, was not significantly different from controls in either caudate (TS = 50 ± 1.7, control = 50.3 ± 1.2) or putamen (TS = 52.8 ± 4.8, control = 43.2 ± 5.5). D2 receptor binding, measured by [3H]spiperone, was slightly greater in the striatum (TS = 21.2, control = 58.9 ± 8.4) and putamen (TS = 39.3 ± 5.3, control = 32.3 ± 2.2).

Our data supports earlier proposals of a dopaminergic abnormality within the basal ganglia. However, rather than a specific dysfunction of postsynaptic D1 or D2 receptors, results from postmortem analysis suggest a significant alteration of DA uptake mechanisms.
DEGENERATIVE DISEASE—OTHER: BASAL GANGLIA

461.1
INTERRIGONAL PATTERN OF STRIATAL CHOLINERGIC ENZYME REACTION IN DOMINANTLY-INHERITED OLIVOPONTOCEREBELLAR ATROPHY: POSSIBLE RELATIONSHIP TO FRONTAL LOBE SYSTEM IMPAIRMENT.


We measured the intraregional pattern of the activity of the cholinergic enzyme cholineacetyltransferase (ChAT), the specific cholinergic marker, in striatum of six patients from one family with dominantly-inherited olivopontocerebellar atrophy (OPCA). Previous neuropathological testing of affected members of this family, including the three patients tested in this study, revealed signs of frontal lobe impairment. As compared with the controls (n=11), mean activity of ChAT was severely reduced in OPCA throughout the subdivisions of the caudate head nucleus with the dorsal portion (-81 to -055) being the most affected. In contrast, ChAT levels in the putamen were much less markedly reduced (-26 to -21%). In view of the experimental and clinical evidence indicating that the caudate nucleus may be a critical component of pre-frontal cortical-governed behaviour, we suggest that the severe cholinergic reduction in caudate nucleus of our OPCA patients may contribute to the frontal system impairment observed in this disorder. (Supported by U.S.N.IH #NS20054.)

SYMPOSIUM:

REGULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR EXPRESSION AND FUNCTION.


Nicotinic acetylcholine receptors (nAChR) are structurally and functionally diverse members of the ligand-gated ion channel superfamily of multi-subunit transmembrane proteins. nAChR diversity is partly based on heterogeneity of nAChR subunit-encoding genes and may provide teleologically beneficial functional and regulatory plasticity to a plastic and dynamic nervous system. The participants will address this thesis and will show how contemporary multidisciplinary approaches are yielding insights into cellular and molecular mechanisms that regulate nAChR expression and function.

Lukas will provide an overview of nAChR biology and a synopsis of studies on muscle and neuronal nAChR regulation in model clonal cell lines. Huganir will describe studies on regulation of muscle-like nAChR processing and functional characteristics by serine and tyrosine protein kinases. Merlie will address transcriptional regulation of receptor expression in muscle with specific reference to promoter analysis in transgenic mice. Berg will review work on regulation of neuronal nAChR function and number in autonomic ganglia. Claudio will discuss studies on synaptogenesis and biogenesis of muscle-like nAChR in transfected cells.

EXCITOTOXICITY V

461.2
EFFECT OF STRIATAL QUINOLINATE LESIONS ON KYNURENINE AMINOTRANSFERASE IN THE RAT BASAL GANGLIA.


Kyurenine aminotransferase (KAT), the enzyme responsible for the production of kyrenic acid (KYN), was studied in the striatum and substantia nigra in normal rats, and in animals 2 and 7 days following a unilateral intrastriatal injection of quinolinic acid (QUIN; 50 µg/1 µl). In normal striata, immunocytochemical studies with anti-rat KAT antibodies revealed a preferential astrocytic localization of the enzyme and a small population of KAT-positive neurons, which was dispersed throughout the striatum. 7 days after QUIN injection, the number of KAT-immunoreactive astroglial cells in the stratum was markedly increased as compared to the contralateral side. QUIN-lesioned striata (glutamate decarboxylase activity; -71.6%; 85.7% decrease in enzyme activity after 7 days) showed a 19.922.4% increase in enzyme activity after 7 days (8-6 each). The early decrease is probably due to the loss of KAT-containing neurons while the late increase may be the result of astrocytic proliferation. KAT activity in the substantia nigra ipsilateral to the lesion was unchanged after 2 days but increased to 141.3% after 7 days (8-6 each). Supported by USPHS grant NS 28236.
**EXCITOTOXICITY V**


3-Hydroxyanthranilic acid (3HAA) is a brain metabolite and bioprecursor for the excitotoxic agent, quinolinic acid (QUIN). Mature (21 days) cultures of rat corticostriatal system were incubated in 3HAA (5mM) alone or concomitantly with the NMDA receptor antagonist, D-APH (Imm). Ultrastructural changes in the cultures were monitored for up to 8 h following incubation. At 10 h, cultures exposed to 3HAA alone began to develop post-synaptic swelling and occasional dendritic swelling away from synaptic contacts. By 24 h, however, generalized dendritic and axonal swelling was severe and even some neuronal swelling was evident; by 40 h, cultures showed treatment. In contrast, simultaneous incubation with 3HAA and D-APH induced no appreciable ultrastructural change in the cultures until 30 hours when there was scattered non-specific swelling of elements in the neuropil; no distinct post-synaptic swelling could be identified. By 50 h, wide-spread swelling of neuronal and glial elements was evident, but dendrites, axons and neurons were still recognizable. These studies suggest that 3HAA can cause toxic damage by two distinct mechanisms: one appears to be excitotoxic in character, possibly in vitro production of QUIN, and antagonized by D-APH while the other may reflect oxidative cell destruction. (Supported by USPHS Grant NS-28236.)

**461.4** MK801 INDUCES THE 70KD HEAT SHOCK PROTEIN AND FOS IN THE CINGULATE GYRUS. James V. Sharp, Stephen M. Sagar, and Frank J. Sharp. Dept. Neurology and Physiology, Univ. Cali. and VA Medical Center, San Francisco, CA 94121

MK801, a non-competitive NMDA receptor blocker, has been shown to protect the nervous system from injury both in vivo and in vitro. However, recent studies suggest that this drug may selectively kill some neurons (Olney et al., Science 244:1360; Allen et al., Science 247:251). Because we have proposed that heat shock gene expression (Gonzalez et al., Mol. Cell. Res. 6:93) may be a useful marker of stressed neuronal populations, we have examined the effects of MK801 on the HSP70 expression. 1mg/kg of MK801 given intraperitoneally induced HSP70 and Fos, detected immunocytochemically in many neurons in cingulate and retrosplenial areas of cortex 18h after administration. This is a dose reported to produce reversible vaculization of these neurons. The effects of 5mg/kg of MK801, a dose reported to produce irreversible neuronal injury in cingulate cortex, is currently being investigated.

The data support the usefulness of HSP70 in identifying cells stressed by a variety of insults. This and other data suggests that cells stressed by various insults that are destined to survive express both HSP70 mRNA and protein, whereas lethally injured cells may or may not express the protein.


Domonic acid (Dom), a structural analog of the excitotoxic amino acid, glutamate (Glu), is believed to be the museal neurotoxin responsible for a food poisoning incident in Canada in 1967 that killed some individuals and left others cognitively impaired. Since information pertaining to Dom excitotoxicity is limited, we have evaluated the neuroexcitory properties of Dom in intact hippocampal neurons (and intact hippocampal neurons) and its neuroexcitory properties both in vitro (chick embryo retina) and in vivo (adult rat).

In vitro, the properties of Dom were compared with those of kainic acid (KA), N-methyl-D-aspartate (NMDA), and quisqualic acid (Quis), each of which is a prototypic agonist at a different subtype of Glu receptor. Under voltage clamped conditions, currents induced in hippocampal neurons by Dom were identical to current induced by KA; both displayed a linear current/voltage relationship (in contrast to NMDA currents) and were non-desensitizing (in contrast to Quis currents). Dom currents were not blocked by antagonists currently induced by CNQX, an antagonist of non-NMDA receptors. In the chick embryo retina, Dom induced the same distinctive lesion pattern as KA which differed from the NMDA or Quis lesion. The Dom lesion was blocked by CNQX but not by NMDA antagonists. Subcutaneous administration of Dom (2.5-3 mg/kg) to adult rats resulted in an acute seizure-brain damage syndrome similar to that caused by Glu and analogous to the neurotoxic syndrome observed in the human food poison victims. Supported by T32 ES07006 (GR3), the Klingenstein Fdn., M45469, P01 NS-38330 (CZ); DA0072, AO868, and RSA M45888 (JWO).


Lumbar spinal subarachnoid injection of dynorphin A (DYN) causes ischemia, neuronal degeneration and persistent hindlimb (HL) paralysis in rats. Excitatory amino acids have been implicated as mediators of DYN-induced spinal cord injury. We were interested in the protective effects of competitive and noncompetitive NMDA receptor antagonists, including MK-801, dextrophan, and dextrorphan (DM). However, recent evidence suggests that protective effects of DM in this model might involve binding sites in addition to those associated with the PCP/NMDA receptor complex. DM binds to high affinity sites which are quite similar to the sites identified by prototypic sigma (σ) ligands such as 6-OH-PPP. To address the involvement of DM/s binding sites in neuroprotective mechanisms, we evaluated the effects of several highly selective substituted phenylethylamines (BD 737, BD 738, BD 1008, and BD 1063) on recovery from the persistent motor deficits caused by L4-L5 subarachnoid injections of 20 min of DYN. Immediate preinjection of these compounds (50-200 mol/L) failed to block the HL paralysis acutely induced by DYN; however all were effective in preventing improvements in HL neurological scores by 24 h postinjection. These results indicate a potential usefulness of a receptor ligands in the treatment of CNS injury.

**461.7** ANITCHOLINERGICS PREVENT NEUROTOXIC SIDE EFFECTS OF NMDA ANTAGONISTS J.W. Olney, J. Labbey*, G.J. Wang, M.T. Price. Washington University Medical School, St. Louis, MO 63110.

Although antagonists of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor are potentially useful for preventing neuronal degeneration in certain neurological disorders, treatment of adult rats with these compounds results in neurotoxic side effects consisting of pathomorphological changes in certain neurons of the cingulate and retrosplenial cerebral cortices (Olney et al., Science, 1969). Following low doses, these side effects may be prevented by non-competitive anticholinergic compounds, when administered systemically, effectively protecting cingulate/retrosplenial neurons against the neurotoxic action of the powerful NMDA antagonist MK-801. We have found that the effective anti-cholinergic compounds have significant affinity for both M1 and M2 muscarinic receptors, their order of potencies in preventing MK-801 neurotoxicity being -thioxypenidyl  alpha trimetazidine > procyclidine > benactyzine > diphenhydramine correlates better with their order of binding affinities for the M1 than M2 receptor. Using the chick embryo retina, we determined that high concentrations of these anticholinergic compounds do not interfere with the ability of MK-801 to protect retinal neurons against NMDA toxicity. These compounds, therefore, provide a simple and safe method for eliminating a potentially serious side effect associated with the use of NMDA antagonists as neuroprotective drugs.

**461.8** EXOGENOUS EXCITATORY AMINO ACID NEUROTOXICITY IN VITRO CAN BE INDUCED BY CYCLIC AMP. A. Morandi*, L. Faccio, D. Milani*, A. Leon and B.D. Sapati. Fidia Research Laboratories, 35011 Abano Terme (PD), Italy.

Glutamate, an excitatory amino acid (EAA) that exerts a trophic effect on immature cerebellar granule cells in culture, is toxic at later stages of cell maturation. We observed that some granule cell preparations failed to develop this sensitivity to excitotoxic injury. As a first step towards understanding the nature of this resistance, prolonged pretreatment (18h with db-CAMP or forskolin (PSK)) was found to confer, in a dose dependent way, sensitivity to the neurotoxic actions of glutamate, aspartate and kainate. Shorter treatment times, or addition of db-CAMP only during EAA exposure or afterwards failed to be effective. The neurotoxic action of glutamate in these sensitized cells was blocked by Mg2+, gangliosides GM1 and inhibitors of protein synthesis. Following removal of db-CAMP, the granule cells再次 became resistant to glutamate neurotoxicity after 48 h. These observations may provide new insights into the mechanisms of excitotoxicity and their possible role in human CNS pathologies.
There is evidence that glutamate neurotoxicity mediates hypoxic injury in select neuronal populations. Studies have shown that dissociated cell cultures of neurons are relatively resistant to glutamate neurotoxicity (Wilson and Kriegstein, Soc. Neurosci. Abstr. 13:763.) Because turtle turtle brain is resistant to prolonged periods of anoxia, we have been able to measure delayed neuronal degeneration in adult turtle cortical slices 24 hours after exposure to excitotoxic amino acids. Cortical slices (400 μm) from adult diving turtles were exposed to glutamate (Glu), NMDA, and kainate (KA) for 30 minutes, and incubated for 24 hours. Measurement of LDH released into the bathing media correlated with morphological injury. The LDH values from the dose-toxicity curves for Glu, NMDA, and KA were 2.2M, 18M, and 0.3M respectively. The Glu uptake inhibitor, dihydroketa, reduced the LDH for Glu to 1000M, but three-0H-aspartate blocked Glu toxicity. APV and CNQX were effective antagonists for NMDA and KA neurotoxicity. We conclude that the slice method is a rapid and reliable means of assessing Glu neurotoxicity in turtle cortex.

461.11 HYPERBILIRUBINEMIA IS ASSOCIATED WITH ENHANCED SUSCEPTIBILITY TO EXCITOTOXIC BRAIN INJURY. John W. McDonald, Steven M. Shapiro. Feas S. Blalock, U. of Virginia. Richmond, VA; and Department of Pediatrics, School of Medicine, University of Virginia, Charlottesville, VA. We postulated that hyperbilirubinemia (HB) may be associated with enhanced susceptibility to excitotoxic brain injury. We studied HB in Gunn rats and in congenital HTN Gunn rats. In Gunn rats, HB was associated with increased excitotoxic sensitivity. In the Gunn rat, HB is associated with increased excitotoxic sensitivity.
INTRINSIC ACTIVITY OF TETRAHYDROPYRIDYL INDOLES AT 5-HT\textsubscript{A} RECEPTORS NEGATIVELY COUPLED TO ADENYLATE CYCLASE.


V. Yang* A. N. Martin†, ‡ of Pharmacology & Toxicology and †Dept. of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, AZ 85721 USA.

This study extends work previously undertaken to determine optimal 5-HT\textsubscript{A} affinity and selectivity, using conformationally rigid analogs of SPYSTP itself (Taylor et al., Mol Pharmacol. 34:42, 1988). The series consisted of analogs of 3-[1,2,5,6-tetrahydropyridyl-3-y]indoles (3-TP1P) and 3-[1,2,5,6-tetrahydropyridyl-4-y]indoles (4-TP1P). Most analogs produced complex inhibition curves in the forskolin-stimulated adenylate cyclase assay (DvL+M, Yaghini, J. Pharmacol. Exp. Ther. 238:248, 1986) using male Sprague-Dawley rat hippocampus. These inhibition curves contained a pindolol-sensitive component, suggesting at least partial 5-HT\textsubscript{A} agonist activity. Analogs producing only weak inhibition of FSC were able to reverse 5-HT\textsubscript{D} induced inhibition by varying degrees. The 4-TP1P analogs generally had higher 5-HT\textsubscript{A} binding affinities than the 3-TP1P analogs. Within each group of analogs, it appeared that the higher the 5-HT\textsubscript{A} affinity, the greater the 5-HT\textsubscript{A} agonist activity. Further analogs must be tested to confirm this trend. Analogs with a carbon-bonded group in the 5-indole position did not follow these trends. (Sup. by N16605 and NS01009).

PRE- AND POSTSYNAPTIC 5-HT\textsubscript{A} RECEPTORS EXHIBIT DIFFERENT ELECTROPHYSIOLOGICAL PROPERTIES: I. EFFECTS OF PERTUSSIS AND CHOLERA TOXINS.

P. Bille, A. Lata and C. de Montigny.

Neurobiol Psychiatry Unit Department of Psychiatry, McGill University, Montreal, Quebec, Canada H3A 1A1.

5-HT\textsubscript{A}, autoreceptors located on the soma of 5-HT neurons and post-synaptic 5-HT\textsubscript{A} receptors on the cell body of CA, hippocampal pyramidal neurons are coupled to G\textsubscript{pro} proteins. In the present experiments, the effect of microiontophoretic application of 5-HT onto dorsal raphe 5-HT neurons and onto dorsal hippocampal CA\textsubscript{y} pyramidal neurons was assessed by 1 to 2 weeks following in situ injections of pertussis or cholera toxin (1 \(\mu\)g in 2 \(\mu\)L of saline). At the earliest time point, the 5-HT\textsubscript{A} response of the same post-synaptic neurons to endogenous 5-HT released by the electrical stimulation of the ascending 5-HT pathway was assessed in the same rats, as 5-HT terminals make synaptic contacts on the dendrites of these neurons. Pertussis toxin, which inactivates G\textsubscript{pro} proteins, markedly attenuated the response of 5-HT\textsubscript{A} neurons and that of CA\textsubscript{y} neurons to the microiontophoretic application of 5-HT, but left unaltered the effectiveness of the stimulation of the 5-HT pathway in suppressing the firing activity of the latter neurons. In contrast, cholera G\textsubscript{pro} coupled mechanisms, did not modify the responsiveness of 5-HT\textsubscript{A} to microiontophoretically applied 5-HT, but reduced both the effectiveness of the stimulation of the 5-HT pathway and that of the microiontophoretic applications of 5-HT on CA\textsubscript{y} neurons. These results suggest that: 1) the 5-HT\textsubscript{A}, autoreceptor of 5-HT\textsubscript{A} neurons is coupled to a G\textsubscript{pro} protein but not to a G\textsubscript{q} protein; and 2) there may exist on CA\textsubscript{y} pyramidal neurons intra-synaptic 5-HT\textsubscript{A} receptors on their dendrites which are coupled to a G\textsubscript{q}, but not to a G\textsubscript{q} protein, and extra-synaptic 5-HT\textsubscript{A} receptors on their cell body which are coupled to both G\textsubscript{q} and G\textsubscript{q} proteins.

NEONATAL 5,7-DIHT DELIVERY ALTERS \textit{METINCERINE}-LABELLED 5-HT\textsubscript{A} RECEPTORS IN RAT BRAIN.

M. F. Francesco, Columbia University, New York, NY 10032.

5-HT receptor denervation supersensitivity has been proposed to explain behavioral supersensitivity to L5-HTP in rats with 5,7-dihydroxytryptamine (5,7-DHT) lesions. No upregulation of 5-HT2 binding sites has been found despite supersensitivity to putative 5-HT\textsubscript{2} drugs. In this study, the 5-HT\textsubscript{A} properties of these drugs are involved instead, we measured 5-HT\textsubscript{A} receptors in rats one month after making neonatal 5,7-DHT lesions by intraperitoneal injection. Sites labelled with \textit{Mincerine} (10 \(\mu\)M 5-HT as displacer) showed a distinct regional distribution: frontal cortex \(>>\) hippocampus-lentico-limbic. In the presence of 50 nM spiperone to block 5-HT\textsubscript{2} sites, 31%, 70%, 65%, 70% and 73% of the sites were present respectively. 5,7-DHT lesions upregulated these sites in frontal cortex (+29%) and hippocampus (+25%). There were no changes in Beau in other regions. 5-HT and norepinephrine were unchanged. Changes in Beau parallel 5-HT concentrations measured by HPLC. These data suggest that 5-HT\textsubscript{A} rather than 5-HT\textsubscript{2} receptor denervation supersensitivity may explain enhanced behavioral responses to 5-HT\textsubscript{2} drugs in rats with 5,7-DHT lesions. (Supported by NIH grant 5-K09-N051158 (CIDA), the Mycologous Research Fund, the United Cerebral Palsy Education and Research Foundation (R381-88), and the William Randolph Hearst Foundation).

ISOLATION, PURIFICATION TO HOMOGENEITY AND SEQUENCE OF A BRAIN ENDORCID FOR \textit{KETANSERIN} RECOGNITION SITES (KBR).

L.A. Aparo*, A. Guidotti, Y. Io, B. Martin, M.L. Barbacina and E. Costa, FGIN, George Washington University, Washington, D.C. 20007; §Laboratory of Biophysical Chemistry, NHLBI and \#Laboratory of Molecular Neurogenetics, NIH, Bethesda, MD 20892.

A peptide that selectively displaces \textit{Ketanserin} binding from crude synaptic membranes has been recently extracted, purified to homogeneity and sequenced. Two major fragments, of calculated molecular weight 40,000xg, showed no significant homology with the A chain or the C chain of the \textit{Ketanserin} receptor, or any other known molecules. However, in a limited region of the \textit{Ketanserin} receptor, changes in the sequence of the hydrophobic portion of the \textit{Ketanserin} receptor, which is involved in the binding of \textit{Ketanserin} to the receptor. The activity of the \textit{KBR} on platelet aggregation and on phosphonolipid hydrolysis in rat cerebral cortex indicate that \textit{KBR} interacts with the 5-HT 2 receptor domain.
462.9

AFFINITIES OF THE TWO ENANTIOISOMERS OF FLUCOXETINE FOR SUBTYPES OF SEROTONIN (5-HYDROXYTRYPTAMINE, 5HT) RECEPTORS.


The two enantiomers of fluoxetine exhibit similar pharmacology as SHT uptake inhibitors, with eudismic ratios near unity (Wong et al, Drug Dev. Res. 6:397, 1985). The R and S enantiomers and racemic fluoxetine inhibited SHT uptake in human platelets at 10^-6 3.6, 4.5 nM (IC50), respectively, but have relatively weak affinity for SHT-1 (A,B,D) and SHT-2 receptors, as indicated by the micromolar IC50 to inhibit binding of radio-ligands to these receptors. However, the R enantiomer and racemic fluoxetine inhibited [3H]muscarine binding to SHT-3 receptors, with IC50 values of 200 and 450 nM, respectively, while the IC50 of S-fluoxetine was 17,000 nM. Fluoxetine and the two enantiomers inhibited [3H]quipazine binding to its recognition sites (Schmidt & Peroutka, Eur. J. Pharmacol. 163:397, 1989). Thus, S-fluoxetine, besides being a potent SHT uptake inhibitor, has an intermediate affinity for SHT-3 receptors. The effects of racemic fluoxetine appear to be mediated primarily via inhibition of SHT uptake, but further studies should determine whether occupancy of SHT-3 receptors is reflected in any pharmacological effects.

462.11


Two 5-HT receptor subtypes, 5-HT1P and 5-HT3, have exocytotic effects on enteric neurons. The 5-HT1P receptor mediates a prolonged depolarization associated with an increase in input resistance. The 5-HT3 receptor mediates a transient depolarization during which the input resistance decreases. Polyclonal anti-idiotypic antibodies (anti-id A8) have been raised by immunizing rabbits with affinity purified antibodies to 5-HT. The crude anti-id A8 were purified by affinity chromatography and shown by immunocytochemistry to bind to a subset of enteric neurons. Anti-id A8 were applied by microinjection to guinea pig myenteric neurons and found to mimic both 5-HT1P and 5-HT3-mediated responses to 5-HT. Responses to the anti-id A8 were antagonized by desensitization of 5-HT1 receptors and by zacopride (BRL 24924), which antagonizes responses mediated by 5-HT1 or 5-HT3 receptors. Following application of anti-id A8, both 5-HT1P and 5-HT3 responses to 5-HT were inhibited (5-HT1P > 5-HT3). This inhibition appeared to be specific because the anti-id A8 did not affect responses to carbamol or substance P. It is concluded that the anti-id A8 binds to 5-HT receptors. Its affinity appears to be greater for 5-HT1P than for 5-HT3 receptors. Supported by grants NS 12969, MH 37575 and the PMAF.

463.1


Previous studies on a variety of neural systems have demonstrated that growth cone morphology is position-specific, becoming more complex in regions where fibers change direction or sort out among themselves. Our analysis of Dil-labeled axons in the mouse optic chiasm (Godement et al., Neuron, in press) show that within this decision region, the most complex growth cones develop on uncrossed axons as they turn sharply toward the ipsilateral optic tract. To link morphology with behavior, we observed growth cones extending in real time. A semi-intact preparation including retina, optic nerves and a slab of ventral diencephalon, was dissected from mouse embryos at E14-16, small injections of Dil in one optic nerve preparation kept in culture for two days. Growth cone behavior was monitored over several hours using low levels of incident fluorescent illumination and time-lapse video recording. Many growth cones advance steadily at rates of 15-50 microns/hr, while others stall, retracting and regrowing repeatedly. Growth cones rapidly change shape, becoming longer with fewer filopodia when advancing, and spreading with more filopodia, when retracting. Retraction and regrowth is often associated with slight shifts in directionality of the growth cone. Thus, growth of retinal fibers in their early environment is a dynamic process, involving changes in form, salutary growth, and process retraction. This analysis should indicate the role of such growth cone behaviors within pathways of growth and in the selection of appropriate trajectories within decision regions. (Supported by NS 27615).

463.2


As mouse retinal axons navigate through the optic chiasm, axons projecting to the contralateral side of the brain cross through the chiasm midline. Preactivated axons initially travel with crossed fibers, but turn back abruptly at the border of a 100 micron wide zone along the midline (Godement et al., Neuron, in press). As uncrossed fibers turn, their growth cones become highly complex. To examine the structure of the chiasm midline, we immunostained the chiasm during the period of axon growth (E14-17) with a number of glial and neuronal cell-surface molecules. Antibodies to NC1, an antigen in immature myelin, stained the CNS (Mason et al., Dev. Br. Res. 44:95, 1989) stained a palisade of radial fibers extending from the floor of the third ventricle through the chiasm to the lateral thalamus. Points of advance of the fiber complex are toward the lateral thalamus, small cells with short processes were also stained. To understand the cell-cell relations of retinal growth cones in the chiasm, we examined the distribution of midline glial fiber bands that were photoduced and processed for EM. Growth cones on straight-growing fibers fasciculated on bundles of other axons. The complex growth cones on turning fibers contacted multiple processes and abutted cell bodies of unidentified cells. Clear and coated vesicles were prominent in the turning growth cones and in the profiles they contacted. This analysis should aid in identifying the cues for crossed and uncrossed fiber navigation, and may implicate properties intrinsic to each of these fiber populations that underlie the differential response to these cues. (Supported by NS 27615).

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
643.2 PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS X THURSDAY 7

643.3 AXON NAVIGATION AT THE MAMMALIAN OPTIC CHIASM: DIRECT OBSERVATION USING FLUORESCENT TIME-LAPSE VIDEO MICROSCOPY. D.W. Sretavan. Laboratory of Neurobiology, Rockefeller University, NY 10021.

Developing mammalian retinal ganglion cell axons reach their optic chiasm and extend to the projecting site of the embryonic CNS. To do so, the retinas, optic nerves together with the chiasms and tracts were isolated from E13-E18 mouse embryos. Dil was deposited into the retina in label ganglion cell axons and preparations were fixed at 37°C or an inverted microscope. Time lapse images of axons were obtained every 30-60 seconds up to 12 hours using a CCD camera.

Several axonal behaviors were seen at the optic chiasm. Retinal axons entering the ipsilateral optic tract often did so by making gradual 90° turns while maintaining continuous growth cone activity. On the other hand, some axons avoided entering the ipsilateral tract by extending a new process in a different direction. Episodes where axons belonging to the two eyes encountered each other often resulted in a passive pause in growth followed by the reemergence of axons in a new direction upon recovery. This behavior was seen only in a subset of axon-axon encounters, suggesting that these repulsive interactions occur between axons from specific regions of the two retinas.

Unlike conventional histological methods, this preparation allows the examination of dynamic interactions between retinal axons and their environment and thus may help in furthering our understanding of axon guidance mechanisms. I thank Torsten Wiesel for encouragement and continued support, and Larry Katz and his laboratory for expert advice on video microscopy and the use of equipment.

643.5 AVAILABILITY RESPONSE AND LOSS OF FISH TEMPORAL RETINAL GROWTH CONES UPON CONTACT WITH CAUDAL TECTAL MEMBRANES OF EMBRYONIC CHICK

C.A.O. Staermeyer, N. Bastmeyer, J. Viehmann. Friedrich-Miescher-Lab./Max-Planck-Gesellschaft, Tübingen, FRG

Caudal tectal membranes of embryonic chick (Walter et al., 1987) and caudal tectal membranes of adult goldfish (Viehmann et al., 1990) contain a repellent guiding component for retinal axons. Temporal retinal axons of fish respond to both, the repellent component of fish and that of E9 chick. The chick component, however, exerts a stronger repellent influence on fish temporal axons than that of fish.

We observed the avoidance behavior of fish growth cones upon contact with chick caudal tectal membranes in a modified in vitro stripe assay with time lapse videomicroscopy. Axon elongate on 90 μm wide laminin lanes bounded by caudal membranes. Upon contact with caudal membranes on one side growth cones sent up to 20 μm long filopodia and lamellipodia onto the membranes, but retracted them rapidly while the growth cone continued to grow on the laminin lane. At the end of the laminin lane, filopodia and lamellipodia contacted membranes on 3 sides. Here, the growth cones collapsed and the axons retracted leaving thin strands of axon material behind. In control experiments, using control instead of caudal membranes, fish temporal axons grew freely into the membranes. The growth cone avoidance behavior will be demonstrated in a time lapse videosequence.

643.6 DYNAMIC BRANCHING PATTERNS IN OPTIC FIBER TERMINAL ARBORES WITHIN THE TECTUM. N.A. O'Rourke and S.E. Fraser. Dept. of Physiol. & Biophysics, University of CA, Irvine, CA 92717.

Retinal ganglion cell axons project into the optic tectum and branch extensively to form terminal arbors. The optic fibers were labeled with a fluorescent vital dye and visualized within the tecta of live Xenopus laevis tadpoles using a laser-scanning confocal microscope to map the complex three-dimensional structure of the arbors. Observation of the labeled fibers over a period of days has revealed that all the terminal arbor, even those with stable dimensions, were continuously remodeled in response to stimuli. To better characterize the branch dynamics, the arbors were visualized at shorter time intervals. Branches formed either through bifurcation at an axon tip or, more commonly, in the proximal portion of the axon at a branch point of another branch. Two classes of branches were found in the arbor. Short branches of less than 4 microns in length, termed "spikes," exhibited a rapid rate of remodeling and were seldom present for more than an hour. Longer branches showed much slower rates of extension and retraction which varied somewhat from one arbor to the next. Interestingly, the branch growth within an arbor was often restricted to one or two branches at any one time, suggesting that the growth machinery within the axon can be shunted selectively to one area of an arbor during the remodeling process. Because all the arbors were dynamic, even those with stable dimensions and tectal positions, these findings suggest that continual remodeling of arbors may be a universal feature of neuronal projections, even in systems previously thought to be static. (NIH EY05836).

643.7 DENDRISTIC DISTINCTIONS BETWEEN CALLOSAL AND SUBCORTICALLY PROJECTIONS PYRAMIDAL NEURONS DEVELOP FROM AN INITIAL COMMON MORPHOLOGY BY ELIMINATION OF EXCITANT APICAL DENDRITES. S.E. Koester and D.D.M. O'Leary. Dept. of Neurosurgery and Anatomy & Neurobiology, Washington University Sch Med, St. Louis, MO 63110.

In adult rats, two different types of cells, which project to layer 5, have similar dendritic morphology. Dendrites in layer 5 have apical dendrites that arborize extensively in layer 1, whereas layer 2 neurons do not. Dendritic arbors in layer 2 do not extend to layer 1 and are referred to as short pyramidal (Hamburger et al., 1988). J Comp Neurol 272:149; Games & Winer 1988, Hering Res 341). To examine the development of the short pyramidal, we examined DI(1) injected into one cortical hemisphere of a series of rat brains that had been double labeled by selective embroyonic and postnatal ages (E22 = 30). Retrosiditically labeled cells in contralateral cortex were examined in coronal sections several months later. In the embryonic cases, the retrogradely labeled cells are likely to be layer 5 neurons since most superficial cortical cells have not extended an axon into the contralateral cortex. Postnatally, layer 5 can be clearly discerned. At ages ranging from E19 to P4, virtually all deep callosal cells extend dendrites into layer 1. At the earlier ages, many of these dendrites are tipped with growth cones, while at the later ages most branch extensively in layer 1. Although there are some subtle differences among layer 5 callosal neurons, that in general form a neurite glioglia with probability P(Pr(x)) or branches with probability P(Br(x)). The orientation dependence of this axon growth exhibits a height (z) of 5 mm, which is a function of the distance from the soma. This height (z) is referred to the soma and then branch point to the next branch point or to the termination of the axon. The illustratfig tree shows a predicted distribution of derived from one of several possible differential equations. The function of the distance from the soma is given by a decreasing function of x. This means that x is small, P(Pr(x)) is small, but generally increases towards 1. P(Br(x)) increases. Many such derived functions, comapcted by computer simulations of rules for dendritic growth, provide good fits to the collected data. Our model implies that almost all of the variation of the dendritic segments may be attributable to complementary probabilities of elongating and branching which change as a function of distance grown from the last branch point. This has significant implications for understanding the development of branching patterns in dendritic trees.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

We have developed an in vitro system to study the specificity of axon-target interactions. Explants of the major cerebellar afferent systems, pontine nuclei for mossy fibers, and inferior olivary nuclei for climbing fibers, were cultured with purified granule neurons from rat. Explants were dissected from mice at stages when their afferents invade cerebellum (P0 for pontine, and E16-17 for olivary explants) and neurites visualized with the mouse-specific monoclonal antibody M6 (Lund et al., J. Comp. Neurol. 247:429,1986). On laminin or poly-lysine substrates or on monolayers enriched for cerebellar astroglia, both pontine and inferior olivary explants displayed extensive neurite growth. In contrast, pontine explants grown on monolayers of their targets, granule neurons, extended many short neurites which terminated on cells close to the explant. This stop signal is abolished by light fixation of the granule cell monolayer. Granule neurites do not act as a stop signal when plated far from, or on a coverslip above, pontine explants. Together with time-lapse video observations, these results indicate that the stop signal requires living target neurons and is contact-mediated. In addition, the stop signal provided by granule neurons is afferent-specific, at least within the cerebellum. Inferior olivary axons, which innervate Purkinje cells as climbing fibers, elaborate long neurites on granule cells. We are further exploring the specificity of the stop signal by co-culturing both types of afferents with Purkinje cells (Baptista et al., this volume). Supported by NS 16951 to C.M. and NS 08761 to D.B.

463.11 A SPECIFIC METALLOPROTEINASE INHIBITOR HAS TRANSIENT EFFECTS ON ACTIVITY OF NEURITES IN VITRO. J. R. Embryonic CHICK NEURAL RETINA CELLS IN VITRO. Joel B. Sheffield and Erik Deblinger. Department of Biology, Temple University, Philadelphia, PA 19122

The developing neural retina expresses a set of extracellular proteins. When retina cells are cultured on a fluorescent gelatin substrate, growing fibers digest substrate in their paths. We suggest that the proteins are used by the tips of growing fibers to allow them to migrate within the mass of the tissue in vivo. We have examined the effects of an inhibitor, steriimiser 1 of HS-Leu-Phe-Ala (HS-LFA) (Durlach et al., J. Biol. Chem., 1990). Embryonic day 10 retinal cells were dissociated and plated at high density (10^6 cells/cm² of surface area) on untreated plastic or glass substrates in medium containing 10% FBS. On the second day, when processes had begun to grow out, the medium was changed to one containing 10 μM HS-LFA. The behavior of the cells was monitored by time-lapse videomicroscopy. Introduction of the inhibitor caused a cessation of both spike extension and growth cone ruffling, while control media had no effect. The behavior of growth cones was in sharp contrast to that of the rat. At E16-17, which continued in activity throughout. The inhibition of activity continued for between three and five hours, and the growth cones then gradually recovered their motility. By the end of the period, it appeared as if the culture had been unaffected by the treatment. Two other potential inhibitors were tested. Bathophenanthroline sulfonate, an inhibitor that chelates zinc from the active site of metalloproteinasises, was toxic. Recombinant human TIMP (Synergen, Inc.) was inactive. The effects of HS-LFA are consistent with the concept that enzymes play a significant role in fiber outgrowth from neural retina cells.

463.12 AXON TRAJECTORY-SPECIFIC DOMAINS IN THE HINDBRAIN OF THE CHICK. Lisa M. Glintner, Dept. of Physiology, Univ. of Odense, Odense, Denmark

The spatial relationships among brainstem neurons that project axons along different trajectories was determined by injecting retrograde tracers into developing axon tracts in 4-8 day chick embryos. Neurons projecting along a specific trajectory were in general organized into one or a few coherent clusters, with the clustering pattern being characteristic for each rhombomere. Injecting different fluorescent tracers into appropriate tracts allowed direct comparison of ipsi- and contra-projecting clusters of reticulospinal, vestibulospinal, and vestibulo-ocular neurons. This showed that such clusters occupy different, often segregated domains, indicating a spatiotopic relationship between soma position and axon trajectory. Whether soma position is involved in determining axon trajectory remains to be seen. Lineage analysis is being performed to determine the extent to which clonal relationships and migration patterns contribute to the formation of trajectory-specific domains.

463.13 THE POSITION OF THE MOTOR NERVE TRUNKS TO THE EMBRYONIC ABDOMINAL MUSCLE IN XENOPUS IS DETERMINED BY SOMITE-DERIVED CELLS. Kathryn Lynch, Uniformed Services University, Bethesda, MD 20814-4799.

It is not yet known what determines the course of major vertebrate nerve trunks. I have used microscopy (EM and LM) to examine the influence of myogenic cells on the distribution of the first motor nerve trunks to the embryonic abdominal muscle (EAM) in Xenopus. The first motor axons arise from spinal motor nerves 2-9, and follow clusters of somite-derived myogenic cells that migrate ventrally between epidermis and lateral plate. At N&F St 39-40, the cells in each cluster fuse to form a segment of the EAM. At St 40, trunks of axons are apposed to the boundaries between muscle segments. Because the myotube tips stain for AChE, the narrow boundary zones are visible in AChE-reacted embryos, normally as six dark transverse lines across the broad flat muscle. However, if a premigratory cell cluster is partially or wholly ablated, the regular segmentation of the muscle is disrupted; an intersegmental boundary may be missing or intermittent, with short sections at different rostrocaudal levels. Nerve trunks in such cases are still closely associated with the boundary zones. When a boundary is missing, two trunks may converge on a single boundary. Nerve trunks zigzag between sections of the intermittent boundary zones. Thus the position of the first nerve trunks is determined by the location of the segmental boundaries. This in turn seems to be a function of the somite-derived myogenic cells, not the environment into which they migrate. Nerve trunk position in older embryos and after ablation of more than one premigratory cell cluster will also be examined.
DEVELOPMENT OF EXCITABILITY IN DISOCIATED CAT RETINAL GANGLION CELLS. 1. Skalnik*, L.M.Chalupa and R.P. Scobey. Depts of Psychology, Neurology and Neurobiology, Graduate Group, Univ. of California, Davis, CA 95616.

We are studying the development of excitable membrane properties in acutely isolated retinal ganglion cells from fetal and postnatal animals. Cells are enzymatically dissociated in a papain solution and kept in suspension at 10°C for up to 48 hours after experimental removal. Ganglion cells are identified by retrograde labeling with rhodamine borne into the LGN and SC. The whole cell and the perforated patch variations of the patch clamp technique are used to obtain voltage and current clamp recordings respectively.

Cells from fetal animals (1 week of age) invariably manifest spikes in response to depolarizing steps of current, and generate sustained- and transient-type firing patterns. At embryonic day 40 (E40) and three postnatal (Kahn & Sur, Soc. Neurosci. Abst. 14-160, 1988), at postnatal day 14, retinal kits were chronically infused for one week with one of the NMDA antagonists, APV (0.5 mM - 1.6 mM), MK-801 (1.23 mM - 4.74 mM), or CPP (0.063 mM- 79.3 mM). Control animals received infusion of saline (0.9%). Compounds were administered via an intracranial cannula (1µl/h), by infusion of a saline solution in a cannula inserted into the brain ventral and medial to the LGN and attached to the minipump (APV, saline). Intracranial injections of H9/YPOA-HPA (209.5%) at three weeks of age reduced the APV and MK-801 prevented retinal affrents from segregating into ON/OFF sublamina. CPP disrupted segregation to block complete separation of sublaminae.

Labeling of single axons in vitro in CPP treated animals showed that in addition to incomplete sublaminal restriction of retinal axon arbors, short side branches had sprouted along the main trunk of the axon. Normally, side branches are seen in the first postnatal week, but have disappeared by two weeks of age. These results suggest that NMDA receptors mediate activity-dependent segregation in the retinogeniculate projection. Since NMDA receptors are crucially involved in retinogeniculate transmission, the effects of NMDA blockade may be a consequence of blocking transmission in the LGN during development.

Supported by EYO 3991 from the NIEL

DEVELOPMENT OF EXCITABILITY IN DISOCIATED CAT RETINAL GANGLION CELLS. 1. Skalnik*, L.M.Chalupa and R.P. Scobey. Depts of Psychology, Neurology and Neurobiology, Graduate Group, Univ. of California, Davis, CA 95616.

We are studying the development of excitable membrane properties in acutely isolated retinal ganglion cells from fetal and postnatal animals. Cells are enzymatically dissociated in a papain solution and kept in suspension at 10°C for up to 48 hours after experimental removal. Ganglion cells are identified by retrograde labeling with rhodamine borne into the LGN and SC. The whole cell and the perforated patch variations of the patch clamp technique are used to obtain voltage and current clamp recordings respectively.

Cells from fetal animals (1 week of age) invariably manifest spikes in response to depolarizing steps of current, and generate sustained- and transient-type firing patterns. At embryonic day 40 (E40) and three postnatal (Kahn & Sur, Soc. Neurosci. Abst. 14-160, 1988), at postnatal day 14, retinal kits were chronically infused for one week with one of the NMDA antagonists, APV (0.5 mM - 1.6 mM), MK-801 (1.23 mM - 4.74 mM), or CPP (0.063 mM- 79.3 mM). Control animals received infusion of saline (0.9%). Compounds were administered via an intracranial cannula (1µl/h), by infusion of a saline solution in a cannula inserted into the brain ventral and medial to the LGN and attached to the minipump (APV, saline). Intracranial injections of H9/YPOA-HPA (209.5%) at three weeks of age reduced the APV and MK-801 prevented retinal affrents from segregating into ON/OFF sublamina. CPP disrupted segregation to block complete separation of sublaminae.

Labeling of single axons in vitro in CPP treated animals showed that in addition to incomplete sublaminal restriction of retinal axon arbors, short side branches had sprouted along the main trunk of the axon. Normally, side branches are seen in the first postnatal week, but have disappeared by two weeks of age. These results suggest that NMDA receptors mediate activity-dependent segregation in the retinogeniculate projection. Since NMDA receptors are crucially involved in retinogeniculate transmission, the effects of NMDA blockade may be a consequence of blocking transmission in the LGN during development.

Supported by EYO 3991 from the NIEL


Spontaneous electrical activity in retinal ganglion cells during early development is thought to play a key role in guiding the formation of visual connections. This activity is essential for the progressive segregation of retinal axonal terminals into eye-specific layers in the lateral geniculate nucleus (LGN) using multi-electrode recording technique, we previously established that cells in isolated immature cat and ferret retinae fired action potentials in bursts that occurred almost simultaneously, and that this activity frequently spread across the piece of retina as a wave travelling at about 100 m/s (Meister et al., Invest. Ophth. 30, 1615, 1991).

In contrast, there were no distinct bursts or wave-like activity in the adult retina. Rather, adult cells showed continuous spontaneous firing in darkness and gave brisk ON and OFF responses to light. We found that spontaneous activity was more prevalent in the retina of young animals (0.6 days of age) than in the retina of older animals (4.6 days of age). In further studies, periodic bursts were also recorded intracellularly from cells in immature retinae which were subsequently identified as retinal ganglion cells by dye injection. Thus, it is likely that some of the cells recorded with the multielectrode array are ganglion cells, suggesting that spontaneously generated patterned activity of these cells may play an instructive role in the segregation of their axons in target structures. The mechanism underlying the synchronous bursting activity during development and the factors responsible for its disappearance in the adulthood are currently being investigated.

(Supported by PHS grant EYO1543 to D.B., NSF grant BNS 8919508 to C.J.S., H.H. Whitney Foundation and L.P. Markey Charitable Trust fellowships to M.M. and an NHMC&G C.J. Martin fellowship to R.O.L.W.)

Individual geniculocortical Y-axons in the cat increase the extent of their innervation within the area from levels of 18 between postnatal weeks of age and adulthood. During this same period, the individual boutons enlarge, increase their spacing and by adulthood have become distributed over an area of 2/3 of the postnatal weeks of age and adulthood. However, the individual boutons enlarge, increase their spacing and by adulthood have become distributed over an area of 2/3 of the postnatal weeks of age and adulthood. The results suggest that prominent intracortical circuits are formed after birth in parietal and ventral visual areas.

Parvalbumin, a calcium-binding protein, was expressed in about 70% of GABA neurons in the mammalian cerebral cortex. Although its exact function is not yet known, PV may effect firing patterns or levels of excitation in fast-spiking types of GABA neurons. One of the main uses of this marker is for PV-immunoreactive (PV-ir) cells in the retina. We have used immunocytochemical staining to study the expression of PV in retinal area 17 in normal and dark-reared kittens. PV was localized with a monoclonal antibody generated against carp muscle parvalbumin (mAb VC1.1) and the lectin VVA. On the day of birth (P1), PV-ir cells were restricted to layer 6 and the subplate zone. By P14, PV was expressed in layers 4-6 and in the subplate. By P21, PV-ir cells were present in all cortical layers except layer I. During subsequent maturation, numbers of PV-ir cells increased in layers 3 and 4 and decreased in layers 5 and 6.

In dark-reared kittens, the number and intensity of PV-ir cells was markedly reduced, however intermediates exhibited normal patterns of PV expression when exposed to light for 4-10 days, after dark-rearing. Dark-reared kittens also showed deficits in receptive field properties but light exposure resulted in recovery of normal receptive field properties. These findings raise the possibility that in dark-reared animals, deficits in receptive field properties in area 17 may be related to lower levels of PV in the basket cells. (Supported by the Klingenstein Foundation.)

CIRCUITRY AND PATTERN GENERATION III

465.1 MEDULLARY SLICES THAT GENERATE RESPIRATORY OSCILLATIONS IN VITRO. Jeffrey C. Smith, John I. Greer and Jack I. Feldsmann, Systems Neurobiology Lab, Department of Kinesthetics, UCLIA, LA, CA 90095-1586.

We identified a medullary slice region of the ventromedial medulla that provides respiratory rhythm (Smith et al., Soc. Neurosci. Abst. 15:955, '89). This region, called the preBötzinger region, was identified by combining extracellular recordings and precision microstimulation of neonatal rat brainstem-spinal cord preparations in vitro. To further test the hypothesis that the region contains cell populations for rhythmogenesis, we cut medullary slices to determine if this region in isolation can spontaneously generate respiratory oscillatory. Intracellular microelectrode and extracellular cord preparation recordings were made some slices mounted in a vibratome bath (bath), sectioned to within 150 μm of the rostral boundary of the critical area, and a 400-500 μm thick transverse medullary slice containing the critical region was cut. The slices preserved rostral portion of the central grey and hypoglossal nerve, allowing recording of oscillatory motoneuron activity in addition to motoneuronal activity within the slice itself. These slices contained the critical region and adjacent reticular formation regions, and excluded all other medullary regions hypothesized to contain cells involved in rhythmogenesis (e.g. Ominski et al., Brain Res 445: 314, '89). The slices spontaneously generated a rhythmic motoneuronal output, demonstrating that they contained network sufficient for both rhythm and (p)neurotransmitter discharge pattern generation. By retaining or excluding different amounts of the flanking regions at the slice boundaries, we obtained evidence for the following features of the spatial organization: (1) There is a cell population(s) at the rostral boundary of the critical region that provides an excitatory drive to the rhythm generating cells essential for the production of oscillatory activity; slices without these cells did not generate a spontaneous oscillation unless cells were depolarized by excessive neurotransmitters, particularly excitatory amino acids acting at non-NMDA receptors. (2) The area bordering the caudal boundary of the critical region is necessary for motoneuronal burst pattern formation. This data is consistent with our model for pattern generation in which there are two functionally and spatially segregated components: (i) a cellular oscillator consisting of conditionally bursting pacemaker neurons requiring excitatory synaptic drive for rhythmogenesis; (ii) separate more caudally distributed populations involved in burst pattern formation that transform the rhythmic drive from the oscillator into the appropriate temporal sequences of action potentials necessary for rhythmic neuronal activity. Supported by NIH grants HL 40959 and HL 02204.

465.2 TIME SERIES ANALYSIS OF SPIKE PATTERNS IN CULTURED AND SIMULATED SMALL NEURONAL NETWORKS. J.M. Kawalski, G. Albert and G.W. Gross*. Dept. of Physics, Dept. of Biological Sciences**, Center for the Study of Biological Complexity, Univ. of North Texas, Denton, TX 76203.

Small neuronal networks (100-500 neurons) grown from dissociated mouse spinal cord as monolayer cultures on a phototouched multielectrode matrix can be maintained for several months and monitored continuously for several days. These networks exhibit spontaneous, highly patterned activity with extensive coarse-grained synchrony. Assuming that the underlying system's dynamics is deterministic, one may apply the Taken's embedding theorem to reconstruct the dynamics of activity and estimate their correlation dimension. We discuss the relevance of this method in the network setting, where the recorded variables are automatic functions of the network state. It is stressed that the presence of a scaling law alone with small exponent for the correlation dimension cannot be considered as a "proof" that the underlying time series has a deterministic origin. Some other important "caveats" in the method's application are discussed (elicitor of the representative variable, error bounds, etc.). We model our networks as ensembles of coupled (via slow variables) Chay's neurons, where each unit may exhibit a full activity range (i.e. resting, periodic, quasiperiodic, and chaotic states). Observed network self-ignition and synchronization phenomena can be explained within this model. Calculated correlation dimension of such models is compared with those from experimental data.

Supported by the State of Texas Advanced Research Program and the Hillcrest Foundation of Dallas, TX, founded by Mrs. W.W. Caruth, Sr.

465.3 SEROTONIN ENHANCES SYNAPTIC FATIGUE IN NEURONAL CIRCUITS OF THE LEECH. P.A. Mangan, P. Dwyer and W. Otto. Department of Biology, Univ. of Virginia, Charlottesville, VA 22901.

Microelectrode concentrations of serotonin (5-HT) elicit swimming activity in isolated leech ventral nerve cords in both normal animals (Williard, J. Neurosci. 1:956-964, 1981) and animals in which endogenous serotonin has been depleted by treatment with reserpine (Frisone et al., Soc. Neurosci. Abst. 14:2384, '88). Previously, 5-HT was reported to modify synaptic transmission between DI motor neuron 102 and central swim oscillator cell 115 (ibid.). We report here serotonin-induced modulation of synaptic physiology in three motor neuron pairs of the leech swim circuit. The synapses examined were those between DI 1 and DE 1 cell 3, VE cell 2 and VE cell 4, and cells 1 and 2.

Inhibitory transmission was monitored prior to and following addition of 50 μM serotonin, in both untreated and reserpine-treated animals. The pre-synaptic cell was injected with depolarizing current pulses (1-3 nA, 1-2 s) and inhibitory post-synaptic potentials (IPSPs) were recorded, digitized, and averaged. Synaptic fatigue increased substantially with 5-HT application in both untreated (30-fold) and reserpine-treated (80-fold) preparations as compared with controls (untreated, reserpine-treated, normal saline; pooled data from all synapses). Serotonin-induced fatigue was 30% greater in untreated preparations than in reserpine-treated preparations. Half-maximal depression was attained in the 0.33, 0.50 and 0.66 in untreated, reserpine-treated preparations, respectively. The increase in synaptic fatigue induced by elevated 5-HT could facilitate oscillatory activity in the reciprocally inhibitory interactions prevalent in the whole central swim network and thus provide the basis for serotonin-enhanced expression of swimming activity in the intact animal. Supported by grants NS08781 (P.S.M.) and NS21778 (W.O.F.)


The feeding central pattern generator of Helixoma trivolvis consists of three subunits (S1-S3) that are independent interneuronal oscillators. These subunits provide excitatory drive to feeding motor neurons. Most feeding-related neurons receive post-synaptic potentials from the S2 subunit. Neurons that receive S2 EPSPs are hyperpolarized by bath application of glutamate or quisqualate, and neurons that receive S2 EPSPs tend to be depolarized by bath application of glutamate or kainate. Glutamate and kainate also stimulate robust patterned motor activity. The compound alpha-amino pimelic acid has both glutamate agonist and antagonist effects in this system. It elicits high frequency patterned motor activity, while gradually decreasing the amplitude of S2-driven pps. This work supports the hypothesis that the S2 interneurons are glutamatergic, activating kainate-like receptors at excitatory synapses and quisqualate receptors at inhibitory synapses. (Supported by NIH Grant NS26145)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
465.5 CRUSTACEAN CARDIOVASCULAR PEPTIDE (CCAP), A HORMONE NEUROMODULATOR OF THE STOMATOGASTRIC NERVOUS SYSTEM. Lawrence J. Martin, Sybil T. Lockhart, and Eve Marder. Biology Department, Brandeis University, Waltham, MA 02254. The nonapeptide crustacean cardiovascular peptide (CCAP) was isolated and sequenced from the pericardial organs (PO) of the crab Cancerurus maenas (Stangier et al., PNAS (1987). We used a polyclonal antibody raised against synthetic CCAP (Driksen & Keller, Cell Tissue Res. 254:347, 1988) to study CCAP-like immunoreactivity in the crab Cancer borealis and the lobster Homarus gammarus. An antibody stained many nerve fibers and terminals in the PO, but no staining was seen in the stomatogastric nervous system. In contrast, in P. interruptus, a pair of cells, or one in each commissure ganglion (CG), showed CCAP-like, as immunoreactivity. Punctate varicosities, resembling neuropil-like structures, were stained in the lateral portion of each CG, as was an unmyelinated fiber within the commissural connective. A single large cell shows CCAP-like staining in the esophageal ganglion.

Preliminary physiological experiments show that bath application of CCAP to the in vitro stomatogastric nervous system produces an ensemble of effects on the motor rhythms controlled by this portion of the nervous system, which controls the muscles of the foregut. Motor pattern changes were induced in both P. interruptus and C. borealis with 1 μM CCAP or less. These results suggest that a CCAP-like peptide may act as a neuromodulator of the central pattern generator of the crustacean stomatogastric nervous system. Supported by NIH Grants NS08543 (LIM) and NS17813 (EM).

465.7 IONIC CURRENT FINGERPRINTS IN LOBSTER STOMATOGASTRIC NEURONS. D.K. Hartline, D.V. Gassle, B.A. Tomiyasu and B.R. Jones. Biokem Department of Neurobiology, Univ. of Hawaii, Honolulu, HI 96822. Two-microelectrode voltage-clamp recordings were made in lobster (0.5 kg Panulirus argus, 22°C) stomatogastric neurons to determine if unique ionic current patterns are associated with different unidentified cell types. Standard test pulses to 0 mV (with and without conditioning prepulses) revealed three outward current (A), and a calcium-dependent inward current (B) composed of fast (C) slow (D) and maintained (E) kinetic components. Current magnitudes in intact and ligated somata are statistically indistinguishable within a cell type. The pyloric group differs from most of the gastric group by having faster A inactivation and slower B inactivation. Differences with the gastric group are shown by differences in A, J and total outward current. Figures show cell specific current patterns (magnitude of A at magnitude of D) for pyloric (left figure) and gastric (right figure) neurons. Possibly significant deviations from cell-specific profiles occur.

Modeling the neurons and outward current signs reduce the space-clamp error (~5-15% at 0 mV) in intact somata. Supported by NIH NS 15314.

465.9 MODELING STUDIES INDICATE THAT SUBTHRESHOLD CURRENTS INTERACT TO SET THE FREQUENCY OF THE NEURAL OSCILLATOR CONTROLLING HEARTBEAT IN THE LEECH. F. De Schutter and R.L. Calabrese. Born Bunge Foundation, University of Antwerp, Belgium and Department of Biology, Emory University, Atlanta, GA 30322. A central neural network generates the motor outflow that programs beat timing of the hearts in the medicinal leech. Two pairs of rhythmically active HN interneurons located in the third and fourth segmental ganglia of the ventral nerve cord form the core of the neural oscillator. The core ionic current of this network is coupled by reciprocal inhibitory synapses and is capable of independent oscillation. Oscillation arises from the interaction of synaptic inhibition and specific ionic conductances. A computer model of one such HN pair was constructed to examine this interaction. It contains equations for fast and slow Ca2+ current, post synaptic current which depends on presynaptic Ca2+ and the delayed rectifier and an hyperpolarization activated inward current (ih) derived from voltage clamp studies in the HN neurons. Equations for slow Na+ current and A current (kA) were derived from the literature. When ih is blocked in in vitro experiments, HN cells stop bursting (Angstadt et al., J. Neurophysiol. 3:845, 1969. This happens in the model but only when a strong A current is present. The actual bursting frequency is determined by the interaction of these two subthreshold currents h and A.

465.6 TWO TYPES OF IDENTIFIED MODULATORY NEURON SUBSERVE TWO TYPES OF FUNCTION: MAINTENANCE OR ALTERATION OF A RHYTHMIC MOTOR PATTERN IN CRUSTACEANS. F. Nagy, B. R. B. P. Campi and A. Moulins. Lab. of Physiological Pharmacology, CNRS, Univ. Bordeaux I, 33270 Arcachon, France. In isolated preparations of the crustacean stomatogastric nervous system, rhythmic activity of the pyloric CPG, and oscillatory properties of its output were found to be strongly dependent upon tonic modulatory inputs from higher centers. A central modulatory neuron (APM) was identified whose discharge can induce rhythmicity in a previously silent pyloric network. However, APM and several other modulatory neurons identified so far are not spontaneously active in vivo, and cannot provide the tonic input to maintain normal pyloric activity. We show in the STG, Homarus gammarus, that the APM function is fulfilled by a par of commissural neurons, P and CP. In vivo, these two neurons are always (n=63) active with the pyloric network. Silencing them provokes immediate cessation of pyloric activity (300 Hz). When deprived of pyloric network, which from cellular oscillators to passive neurons. When compared to APM, P and CP appear to exert significantly different effects. While all three modulators can induce oscillatory properties in previously passive pyloric neurons, only oscillations induced by P and CP resemble normal pyloric activity; whereas a brief discharge of APM at only 10 Hz lastingly transforms the pyloric pattern.

These results, therefore, indicate two classes of modulatory neuron: those like P or CP, whose sustained discharge maintains basic rhythmic activity of a CPG; and those like APM, with strong and longlasting effects but episodic discharge, which induce temporary alteration in the ongoing activity of a CPG.

465.8 USE OF MODELING TO ANALYZE ORIGIN OF EMERGENT OSCILLATIONS OF THE GASTRIC MILL CPG IN THE LOBSTER STG. F.P. Renart and J. A. Mandell. Biology 2-02, U.C. San Diego, La Jolla, CA 92093-0230. The gastric mill CPG oscillates at low frequencies without a pacemaker cell. All component cells and physiological synapses are known, but strengths of tonic and synaptic currents are not well characterized due to limitations of voltage clamp techniques. Most gastric cells display plateau potentials and have conditional bursting properties. On the basis of its behavioral characteristics, we hypothesize that the network has the dynamics of a relaxation oscillator. Modeling and phase portrait analysis are used to analyze how synaptic and electrotonic connections and membrane currents contribute to emergence of network oscillations. Modeling software was developed in which a model runs forever while concurrently allowing variation of all model parameters. As new cells cross the display, the effects of parameter changes are seen immediately. Our strategy is to start with a minimal network model and extend it as forced by the requirement that model behavior match the observed oscillatory behavior. Our present learning algorithms were developed for the adjustment of model parameters to match oscillatory behavior, thus allowing prediction of unseen behaviors. Conversely, if parameter adjustment is impossible, new model currents must be added. The starting point is the Hopfield network model which only includes passive membrane properties and a non-zero, self-regurrent synaptic term, corresponding to a voltage-gated membrane current. Each synapse is modeled by a saturating current. If the product of the self-regurrent synapse’s weight with the slope of the linear portion of its current is greater than membrane leak conductance, plateau potentials appear. Addition of a current with a single control parameter which increases from zero results in a Hopf bifurcation to oscillations with zero frequency. Each model cell must transduce observed input signals to phases of output signal phase. For some cells, notably In1, this requirement forces addition of another current.

465.10 GENERATION OF THE SHORTENING MOTOR PATTERN IN THE MEDICINAL LEECH. G. Winssinger and W. B. Krums. Jr, Department of Biology, University of California at San Diego, La Jolla, CA 92039-0320. Shortening behavior in the leech is on a gross level a simple multisegmental reflex, but detailed analysis of its motor pattern reveals surprising complexities. The gross appearance of the behavior is longitudinal shortening of several segments near the site of mechanical stimulation. However, recordings from motor neurons [MN] involving longitudinal muscle reveal that the segment closest to the stimulus is bending away from it. A single dorsal excitatory MN is inhibitory to the segmental excitatory MN immediately anterior and the ipsilateral ventral excitatory MN is inhibited by the contralateral ventral excitatory MN. A ventral stimulus results in excitation of both ventral excitatory MN the ipsilateral dorsal excitatory MN, and inhibition of the contralateral dorsal excitatory MN. A dorsal stimulus in excites the ipsilateral excitatory MN and excitatory MN to the segmental excitatory MN. Both the inhibitor and excitatory MN to the same target MN, but in accord with von Holst’s max that nervous systems “need the mess just as much as the whip.” This is borne out in the responses of the MN in the two ganglia anterior and two ganglia posterior to the ganglia in which the MN are stimulated: all longitudinal excitatory and inhibitory MN are excited (with a few exceptions), with the result the observed longitudinal shortening. In order to determine the neuronal circuitry responsible for this motor pattern, the connections of several interneurons have been studied. Cell 115, an interneuron involved in both swimming and local bending behavior, plays a role in shortening but excites only dorsal excitatory MN and thus is not sufficient for the whole behavior. Unexpectedly, inhibitory MN contribute to shortening behavior by affecting motor output in adjacent ganglia. For instance, depolarization of the dorsal inhibitory MN causes inhibition of the dorsal excitatory MN in the same ganglion, but causes excitation of the dorsal excitatory MN in the anterior ganglion posterior to it. This effect can be explained by the circuitry of the shortening behavior, in which the excitatory motor neurons of the cell 115. Thus an important role in patterning of the “simple” reflex is played by the motor neurons themselves. Supported by NIMH research grant MH443936 and PHS training grant GM07198.
**CIRCUITRY**

**465.11**

EFFECTS OF SEROTONIN ON SWIM-GATING NEURONS IN THE MEDULLARY LEECH. J.D. Assentat and W.O. Friesen. Department of Biology, University of Virginia, Charlottesville, VA 22901.

Prolonged activity of cells (e.g. cell 204) is an important step in the initiation of leech swimming behavior (Weeks, J.C. and Kristan, W.B., J. Exp. Biol. 77:1, 1978). Previous studies (Willard, A.L., J. Neurophysiol. 39:19, 1976) showed that the probability of spontaneous swimming in intact animals or swim-associated electrical activity isolated in nerve cords is increased by the neurotransmitter serotonin (5-HT). We have examined the effects of 5-HT on the cellular properties of cell 204 in isolated ganglia (G10-G14). Data from ganglia exposed to 50 μM 5-HT for a minimum of 30 min (n = 24) were compared to controls (n = 24). Cell 204 was penetrated with a single microelectrode and voltage responses to 1 sec current pulses (+2 to +2 nA) were measured using discontinuous current clamp. We also measured the amplitudes of postinhibitory rebound (PIR) at the offset of hyperpolarizing current pulses and of hyperpolarizing undershoots at the offset of depolarizing current pulses. The 1-V curves obtained in the presence of 5-HT were identical to controls except for a small increase in the hyperpolarizing responses to -0.5 and -1.0 nA current pulses. However, in the presence of 5-HT, the peak-amplitude of PIR and of hyperpolarizing undershoots were increased by an average of 190% and 330%, respectively. In Na-free saline, the peak amplitude of PIR was significantly reduced compared to controls. In addition, the serotonin-mediated increase in PIR observed in normal Na saline was eliminated in Na-free saline. These data suggest that ionic conductances intrinsic to swim-gating cells are modulated by 5-HT. Supported by NIH grants NS08089 (JDA) and NS21778 (WOF).

**465.13**

NMDA RECEPTORS CONTRIBUTE TO PROLONGED EXCITATION OF THE SCRATCH REFLEX CIRCUIT IN THE TURTLE SPINAL CORD.

Scott N. Currie and Paul S.O. Stein, Department of Biology, Washington University, St. Louis, MO 63130.

Cutaneous stimulation in the turtle produces an increase in the excitability of scratch reflexes that outlast the stimulus by several seconds (J. Neurophysiol. 60:2122, 86). We recently identified 'long-afterdischarge' cutaneous interneurons in the midbody spinal cord that may participate in multisegmental excitability storage in the scratch reflex pathway (Abstr. Soc. Neurosci. IX:1118, 86). The two characteristics of long-lasting excitation demonstrated for scratch reflex motor patterns are also exhibited by long-afterdischarge interneurons: 1) responses can continue for many seconds after a brief cutaneous stimulus and 2) strong temporal summation occurs when single electrical pulses are applied to cutaneous afferents at multisegment intervals. In the present study, we examined the ability of D-2-amino-5-phosphonovaleric acid (APV), a specific antagonist of the N-methyl-D-aspartate (NMDA) receptor, to block prolonged cutaneous-evoked excitation. We applied APV to the exposed dorsal surface of a midbody spinal cord segment that receives cutaneous input from the radial scratch receptive field. We simultaneously recorded single-unit activity from interneurons in the exposed segment and noted activity from hindlimb motor nerves. 50 μM APV strongly suppressed the activation of long-afterdischarge interneurons and completely blocked the temporal summation of scratch motor output when electrical stimuli were applied to the shell at multisegment intervals; it also greatly attenuated interneuron and scratch responses to higher frequency electrical stimulation and constant-force mechanical stimulation. We conclude that NMDA receptors assist in producing the long-lasting excitation that is a critical component of sensory integration in the scratch reflex pathway. Supported by a postdoctoral fellowship from the Washington University Center for Cellular and Molecular Neurobiology to SNC and NSF Grant BNS-8808144 to PSBS.

**466.1**

TRANSCRANIAL DOPPLER ULTRASONOGRAPHY DURING COGNITIVE STIMULATION.

R.C. Kelley, N.J. Tischendorf*, J.Y. Chang*, B.E. Levin*, B.C. Duncan*, S.J. Lee*, Dept. of Neurology, University of Miami School of Medicine, Miami, FL 33101.

Transcranial Doppler ultrasonography was evaluated for its potential to detect selective cerebral activation during cognitive tasks in 21 normal subjects. Mean and maximum flow velocity of the anterior cerebral arteries (ACAs), middle cerebral arteries (MCAs) and posterior cerebral arteries (PCAs) were measured during performance of a commercially available video game with right-handed manipulation of the joystick. We also measured flow velocity of the ACAs in 18 subjects during a mental arithmetic task. Mean and maximum flow velocity of the right ACAs were significantly higher than those of the left ACAs during task performance. During the video game, both MCAs (r=2.67, P<0.08 for the left, r=4.96, P<0.01 for the right) and the left PCA (r=0.13, P=0.04) had selective increases in mean flow velocity compared to the ipsilateral ACA. This selective activation was most prominent in the right MCA where the side to side difference was borderline significant at r=4.16, P=0.05 for mean velocity and r=3.85, P=0.06 for maximum velocity. We did not observe selective activation during the math task. This technique has the potential to allow noninvasive monitoring of circulatory correlates of cognitive activity.

**466.2**

REAL TIME OPTICAL DETECTION OF RHYTHMIC MOTONEURON AND INTERNEURON ACTIVITY IN THE ISOLATED SPINAL CORD USING DIGITAL IMAGING OF CALCIUM TRANSIENTS. Michael O'Donovan, Wayne T. Metz, and Jon S. Feller, Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, Texas 77030.

We have used real time digital imaging of calcium transients to identify active motoneurons and interneurons in the lumbar spinal cord of ES to E12 embryos. Activity was recorded using the Fura-2AM antagonist, which was loaded into the cord with a microinjection electrode and monitored with a high-speed video camera. Activity detected in 120-300ms during episodes of motor activity. The fluorescence (F) of loaded cells was monitored with an intensified video camera, and recorded on video tape together with hindlimb muscle nerve activity. At E12, when antagonist motoneurons alternate, the activity was widely distributed throughout the spinal cord but were concentrated in the lateral motor column (LMC), the intermediate region dorsal to LMC and in the dorsal horn. Fewer active cells were found medial to the LMC. By E16 when the motor pattern is less mature, the active cells were more restricted in their distribution and were concentrated in the LMC and the intermediate region.

During motor activity motoneurons exhibited oscillations of fluorescence (F/F0 = 15-50%) in phase with rhythmic activity recorded from the muscle nerves. Cells in the intermediate region and the dorsal horn, presumed to be interneurons, also showed large fractional changes in fluorescence during motor activity. Some were rhythmically active in phase with motoneurons whereas others were tonic activity throughout the episode. In some experiments the calcium transients have been measured in over 50 cells simultaneously. Experiments are now in progress to characterize the active cells histologically.

**Cortex IV**

**SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990**
646.3

REPRESENTATION OF MOVEMENTS IN PREMOTOR CORTEX OF SQUIRREL MONKEY. EVIDENCE FOR A HOMOLOG OF THE ARCULATE PREMOTOR AREA. K. Nudo, Dept. of Neurobiology & Anatomy, Univ. of Texas Medical School, Houston, TX 77030

We have recently reported that primates possess a unique source of cortical fibers, originating within Area 6 in the lateral portions of frontal cortex of the squirrel monkey and New World monkeys, and buried in the caudal bank of the inferior arcuate sulcus in Old World monkeys (Region C; Nudo & Masterton, 1990). While these results suggest that 6 is continuous with the rostral two-thirds of the arcuate/premotor cortex, recent observations and intracortical microstimulation results indicate that this may not be the case. In particular, a recent study (Connors et al., 1988) found that the rostral extension of Area 6, located ventrally and caudally to the arcuate, contained a region of motoneuronal representation that was independent of Area 6, possibly homologous to the human premotor cortex.

646.4

MICROSTIMULATION MAP OF THE MONKEY PREMOTOR CORTEX. M. Godechkar, A.R. Mitz, J. van der Burg and R. van Duinen, Department of Anatomy, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

Interpretations of premotor cortex (PM) somatotopy differ between the relevant anatomical (Muakkassa & Strick, Brain Res. 177:374, 1979) and physiological (Kurata, Exp. Brain Res. 77:265, 1989) studies. Coarse microstimulation of PM did not resolve this issue (Rizzolatti et al., Exp. Brain Res. 71:141, 1988). A more detailed and complete microstimulation map of the periarcuate and surrounding cortex was necessary.

A large chronic craniotomy was performed over the arcuate (AS), superior precentral (SPCS), and central sulci. Biphasic constant-current pulses of up to 65 μA were delivered along 110-180 tracks in each of 4 macaque monkeys.

Movements were evoked from nearly every penetration into PM. Thresholds were typically below 60 μA, and often below 20 μA. Orofacial movements were evoked from the fundus and the caudal bank of the lateral limb of AS. Arm and hand movements were elicited from the caudal bank of AS, along the genu and the adjacent lateral limb. Forelimb sites continued onto the convexity medially and caudally towards SPCS.

Thresholds increased with distance from AS cortex. Leg movements were elicited from an area around SPCS. Based upon these results, PM contains a whole body representation nearly parallel to MI, not one that follows the curvature of AS.

646.5

CONTRASTING PROPERTIES OF THREE FRONTAL REGIONS (G, di Pellegrino and S. Wise). Lab. of Neuropsychology, NIH, Bethesda, MD 20892

To reveal the physiological differences between prefrontal cortex (PF) and frontal motor areas reflect valid contrasts in their properties or do they result instead from the diverse behavioral and physiological methods used to study them? To address this question, a monkey (Macaca mulatta) was conditioned to begin each trial by recontacting the central three touch pads. Next, a 1-s long red or green stimulus appeared directly in front of the monkey, followed by 1.25 s (the delay period). Then one end and opposite patterns simultaneously appeared, as the red or green stimulus above the potential target. The monkey withheld its response for an instructed delay period of 1.25 s, then touched the pad under the peripheral cue matching the first stimulus. We sampled the dorsomedial, homonotopic PF cortex ventral to and within the principal sulcus (97 neurons), the agranular dorsal premotor area (PM, 55 neurons), and a dysgranular region dorsal to the PM terminal of the arcuate (AS, 76 neurons). The proportion of movement-related neurons was not significantly different among the three regions explored. However, cells with apparent selectivity for stimulus attributes were found only in PF (14%). Further, during the first delay, phasic responses were prevalent in PF and DMP, but absent from PM. During the instructed delay period, phasic discharges predominated in PF (48%) and DMP (78%), whereas in PM fewer cells (26%) showed this type of activity. Conversely, in PM, tonic discharges were most common (60% vs. 9% in PF & 11% in DMP). Over activity and activity duration differed between PM and the other two areas. Thus, we found that the previously reported differences between PF and PM are reliable, and do not reflect species, individual, or methodological variation. In addition, the neural properties of DMP and PM more resemble each other than those of PM.

In a task variation, the green stimulus did not appear on trials in which the correct response was to target beneath the green peripheral light. None of the 6 PM cells tested showed any effect of eliminating the central green stimulus. By contrast, 6 of 9 PF neurons were dramatically affected.

646.6


The aim of the present study was to identify corticocortical and thalamocortical inputs to MI neurons whose activity properties in relation to a learned motor task were examined. Monkeys (Macaca fascicularis) were trained to reach for and push one of two push buttons according to visual instruction signals. The signals were presented for a key for 2 sec, either a right or left push button, placed in a panel facing the animal, was illuminated for 500 ms with an LED from behind the panel. An animal was expected to reach to the key, 2 to 4.5 sec before releasing the key and push the illuminated button. The neuronal activity in MI was classified into two broad categories, the activity immediately preceding the reaching movement (W) and the activity during the instructed wait-period (I). Under general anesthesia and aseptic conditions, stimulating electrodes were chronically implanted into the supplemental motor area (SMA), postcentral cortex (Post) and thalamus (Thal). MI neurons exhibiting only the I response were frequently activated by SMA but less frequently by thalamus stimulation. Hardly any of them were activated by Post stimulation. Neurons with an activity were most frequently activated by Thal and Post stimulation, and less frequently by SMA stimulation.

646.7


Recently, we have identified a previously unknown but sizable direct GABAergic projection from zona incerta to the neocortex in rats (Gutekunst, in press, 1990). This projection is cortically organized and evidence for a bilateral connection. To determine whether this pathway is also present in other species, we measured intrinsic GABAergic tracer fluorosilicate injected in several cortical areas in the monkey. Results of these studies confirmed that this zona incerta-cortical GABAergic projection is also present in New World monkeys (Macaca). Next, to determine if the functional character of the ZI, single units were recorded in the ZI of rats anesthetized with pentobarbital. Since the trigrammatic nuclei are known to project to the ZI, somatosensory mapping studies were performed. In the dorsal subregion of the ZI were found to possess very large receptive fields (RFs) on the fur or whiskers of the contralateral or bilateral face. By contrast, neurons in the more ventral subregions of ZI possessed RFs on the ipsilateral face. Both slowly and rapidly adapting responses were found in ZI. In addition, circular visual RFs were found and both of the following features: Single unit recordings in the general region of ZI in awake, freely behaving rats also revealed neurons with combined somatosensory RFs (whiskers) and visual RFs covering the visual space in front of the whiskers. These cells were maximally active in the same direction of movements of the whiskers when the animal searched the surrounding space or novel objects. Units also responded strongly when an object was rapidly moved towards or away from the monkey. Two units suggested ZI may involve both sensorimotor integration and attention. Supported by grants NS26772, AA04965, K02-000008, AFG000-0286 and PAPES 8644044.

646.8

FIRING PROPERTIES AND SYNAPTIC RESPONSES HAVE INVERTED LAMINAR DISTRIBUTIONS IN REELE N EOCORTEX. B.W. Connors, L. Silva and M. Gutnick. Sect. of Neurobiology, Div. of Biology and Medicine, Brown Univ., Providence, RI 02912

Reeler is a mouse mutation that alters neuronal migration during development, yielding an inversion of the laminar organization of the neocortex. In vitro slices from typical normal and reeler parietal cortex to study the influence of laminar position on neuronal membrane properties and synaptic responses. Dye injections in reeler pyramidal neurons revealed atypical morphologies, including distorted apical dendrites and cell inversion. The intrinsic firing of apical dendrites of reeler cortical neurons was similar in height, resting membrane potential, input resistance and evoked EPSPs and IPSPs did not differ between reeler cortical neurons and normal controls when data were grouped. However, the laminar distribution of intrinsic firing patterns (TINS 13:99, 1990) was inverted in the reeler: intrinsically bursting neurons were found only in layer 5 in the normal mouse, but they were found exclusively in superficial layers of the reeler cortex. The laminar distribution of synaptic responses in the reeler was also inverted: very prominent IPSPs were characteristic of upper layer neurons in the normal mouse, but in the reeler similar responses were observed in deep layer neurons. Deep layer neurons in the normal mouse also exhibited latency and amplitude differences that develop with age, such that neurons in superficial layers are more prominent with age. Supported by the NIH, the Kimmel and the McCormick Fund.
466.9 LAYER 5 NEURONS CAN INITIATE NMDA-DEPENDENT, 4-7 Hz SYNCHRONIZED RHYTHMS IN NEOCORTEX. L.R. Silva and B.W. Conord, Section of Neurobiology, Div. of Biology & Medicine, Brown University, Providence, RI 02912.

We have studied a cellular mechanism of synchronized rhythms in slices of rat SI neocortex in vitro. Slices bathed in Locke's buffer containing zero Mg* show spontaneous synchronized discharges that were highly rhythmic. Field potentials revealed epochs of 4-7 Hz discharges. Each epoch typically lasted 4-4 sec, and recurred at 1-2 Hz per min for hours. Synchronized rhythms were dependent on NMDA receptors, since they were completely blocked by the specific and selective APE. When slices were cut vertically into narrow segments (-1 mm wide), rhythmic spontaneous activity continued in each piece. When segments were then cut horizontally, rhythmic activity continued only in fragments with an intact layer 5. Fragments consisting only of layer 5 were also spontaneously rhythmic. Recordings revealed that layer 5 pyramidal neurons whose apical dendrites were severed at the layers 4/5 border had normal firing properties; the majority could oscillate endogenously at 5 to 15 Hz (cf. Silva et al. Neuron, 15:660, 1998). Since layer 5 was necessary and sufficient to produce rhythmic synchronized discharges in this frequency range, we suggest that some EFG rhythms are generated by a network of endogenously oscillating pyramidal neurons in layer 5.

Supported by the NHI, the MHI, and the McConnell Fund of Stanford University.


Intracellular recordings from interneurons in the rat frontal cortex and parietal cortex were made from slices of zero Mg* perfused with a pH 7.4 (150 mM NaCl, 2.5 mM CaCl2, 1.25 mM NaH2PO4, 25 mM glucose, 25 mM NaHCO3) solution. The membranes of parvalbumin-containing interneurons were visualized with BODIPY. The membrane potential oscillations were identified as 10-50 Hz and were usually considered to be CA8ergic interneurons. Further study of the layer IV neurons revealed that, in addition to the 40 Hz oscillatory interneurons, in which an all-or-none plateau potential generates a close-to-frequency oscillation (35 to 45 Hz), a second category of cells exist where the subthreshold oscillation may vary from 10 to 40 Hz. This second type of neuron responds with subthreshold oscillations of variable frequency, depending on the level of membrane depolarization. Both types of neurons are likely to send inhibitory oscillatory inputs to cortical pyramidal cells as well as to other neurons within a cortical column. This interneurons network may serve to transform dissonantly thalamic input into 40 Hz oscillatory Pyamidal cell firing, triggered as a rebound response to the synchronized IPSP input from the interneurons. This cortical circuit is proposed to be a part of the cellular mechanism for the "branching" or "conjunction" properties presently considered to be a main function of the 40 Hz rhythm in the brain.

NERVE GROWTH FACTORS IX

466.11 LONG-TERM RECORDING OF CORTICAL UNITS USING THE CONE ELECTRODE IN MONKEYS. F.R. Kennedy, R.A. Bakay, N. Otsuji and M.D. Parker, Emory Univ., Atlanta, GA.

Recording the electrical activity of cortical neurons from many years with adequate signal-to-noise ratios will allow patients to control prosthetic devices. Multi-units, or for more selective control, single units must be recorded for years from voluntary motor areas. From cortical area 4, we have recorded multiple single units for over a year in one monkey, and six months in another. From these single units we separated single units that were identified over these periods to movements.

The recording electrode consists of a tiny glass cone with a 100-120° cone angle that is inserted into the skull. The cone angle is critical to limit the spread of the current. The recording electrode is connected to a high impedance amplifier and a multichannel recorder. The entire system is designed to allow stable recordings, well beyond one year.

The monkeys are videotaped while taking food. On review, the single units are separated using the waveform parameters determined at earlier sessions. Individual units are reviewed at a time in synchrony with the videotaped hand and finger movements, thus revealing the relationships between the units and movements.

466.12 TOTAL NUMBER OF NEURONS IN HUMAN NEOCORTEX RELATED TO AGE AND SEX. B. Pakkenberg, S.M. Evans, A. Moller, H. Brasedet, & H.J.G. Gundersen, Neurological Research Laboratory, Bividsve University Hospital, Copenhagen and Stereological Research Laboratory, Aarhus University, Denmark.

A method for an unbiased estimation of the total number of neurons in the human neocortex has recently been introduced and applied to 50 brains from normal individuals of different age-groups and both sexes. The sampling was designed so that the majority of cerebral cortex was left intact providing the possibility for resampling and further analyses. Uniform sampling for total neuron number was performed in each neocortical area. Total cortex volume was estimated precisely according to Cavalieri's principle, and neuronal numerical density estimates made in 35 µm thick plastic sections using optical dissectors. Normal humans have approximately 20 - 10⁶ neocortical neurons with an inter-individual variation of 15%. There is a decline in the number of neurons with age by 50 to 100 - 10⁶ neurons per year. Females have about 15% fewer neurons than males of same age.

INSERM U114, Collège de France, Paris, France.

467.1 DEPOLARIZATION MODULATES CILIARY GANGLION NEURONAL RESPONSES TO NEUROKINES. F. Fuller, A. Les, J. Kloss, B. Cordei, S. Varon and H. Manthorpe, California Biotechnology Inc., 2450 Bayshore Pkwy, Mountain View, CA 94043; Dept. Biology, UCSD, La Jolla, CA 92039.

Neurotrophic agents, including depolarizing concentrations of potassium, prevent the death of cultured chick E8 ciliary ganglion (CCG) neurons. To examine the effects of neurokines independent of their survival activities, we have tested the effects of factors that affect ciliary ganglion cultures maintained in 40 mM K⁺ on a laminin substrate. Without added factors, neurite outgrowth was essentially absent and cholinergic neurotransferases (ChAT) activity decreased with time. However, IGF-1/Insulin, FGF and CNTF were potent stimulators of both ChAT and neurite outgrowth. FGF elicited the largest increase in ChAT (2 to 3 fold), while CNTF was the most potent (Exo-5 g/ml). Under depolarizing conditions (4 mM K+), IGF-1 was not active while FGF and CNTF were both cholinergic and neurite outgrowth of CCG neurons and to prevent loss of ChAT activity. Furthermore, CNTF was 10 fold less potent in low potassium. These observations suggest that, in vivo, the role of depolarization may be to modulate neuronal response to neurokines. Supported by NIH 18349.

467.2 REGULATION OF PROTEIN TYROSINE PHOSPHORYLATION IN DEVELOPING MOUSE BRAIN BY EGF, IGF-1 AND INSULIN. Jean-Antoine Gitis, Gloria Bertuzzi, James K.T. Wang, Dennis Pang* and Paul Greengard, Rockefeller University, New York, NY 10021.

Inulin-like growth factor 1 (IGF-1), insulin, and epidermal growth factor (EGF) have trophic effects on neuronal development in vitro. Since their receptors possess tyrosine kinase activity, we have investigated the effects of these growth factors on protein tyrosine phosphorylation in disaggregated cultures from embryonic mouse cerebral cortex, using anti-phosphotyrosine antibodies. In the absence of growth factor, two main proteins phosphorylated on tyrosine were observed and designated p120 and p110 on the basis of their apparent molecular weights on SDS-PAGE. EGF enhanced the phosphorylation of p110 and induced the appearance of a 110 kDa and a 170 kDa phosphoprotein, the latter probably corresponding to the EGF receptor. Insulin and IGF-I increased the phosphorylation of p120 and of a 160 kDa phosphoprotein distinct from the EGF receptor. Platelet derived growth factor, basic fibroblast growth factor, nerve growth factor and bombesin had no effect on protein tyrosine phosphorylation.

In the brains of intact mice, at various ages from embryonic day 16 to the 4th postnatal week, protein tyrosine phosphorylation was unaffected by 500 ng of EGF, insulin or IGF-I, applied during development in vitro and in vivo, reaching a maximum during the postnatal period, earlier for p120 than for p110. We propose that the phosphotyrosines identified in the present study mediate some of the effects of EGF, insulin and IGF-I in developing cortical neural tissue.
3 REDISTRIBUTION OF FIBROBLAST GROWTH FACTOR AFTER NEURITIC INJURY Timothy J. Neuberger and George De Vries Dept. of Biochemistry, Medical College of Virginia, Richmond VA, 23298

We previously reported the presence of fibroblast growth factor (FGF) in Schwann cells (Sc) and neurons (Nc) in dissociated dorsal root ganglion (dDRG) cultures (Neuberger et al., 1990). In this study we crushed the neuritic field of established dDRG and visualized the distribution of FGF at various times after injury. At 1 and 3 days post-injury (DPI), the distribution of FGF appeared unchanged from the normal control culture; FGF immunoreactivity was observed in the cytoplasm of both Nc and Sc but not in any Dc. However, by 4 DPI, FGF detected on the plasma membrane of the Nc bodies was decreased, whereas FGF associated with the cytoplasm of Nc and Sc remained unaltered. At 4 DPI, the outer membrane of Sc also demonstrated intense FGF immunoreactivity. Most Sc appeared morphologically normal; however, the membrane of a few Sc demonstrated extensive blebbing. At 6 DPI, the FGF distribution was similar to that seen at 4 DPI. In this study, we demonstrate that neuritic injury results in a significant redistribution of FGF; the outer membrane of Sc become positive for FGF while the outer neuronal membrane demonstrates decreased immunoreactivity. The potential role of this redistribution of FGF, in peripheral nerve regeneration is under active investigation. (Supported by NS10821 and NS15408)


Brain-derived neurotrophic factor (BDNF) and neurophin-3 (NT-3), two recently cloned molecules closely related to nerve growth factor (NGF), were produced from human cDNA expressed in human embryonic kidney cells. The recombinant proteins were tested in cultures of dissociated fetal rat brain. BDNF but not NT-3 stimulated the differentiation of basal forebrain cholinergic neurons, similarly effective as NGF (Knipsel, B., et al., J. Neurosci., 10:558, 1990) and as NGF which is well established as neurotrophic factor for these cells. In contrast to NGF and NT-3, BDNF also increased protein content of the cultures. BDNF, as NGF, was early time in-vitro. Maximal increase of choline acetyltransferase activity after 3 days treatment with BDNF was approximately 300% of control. Possible actions of BDNF and NT-3 on dopaminergic neurons of ventral mesencephalon in culture are currently studied. Our findings suggest an important role of BDNF in mammalian brain development with a spectrum of actions different from that of NGF.

7 CILIARY NEUROTROPHIC FACTOR IN ADULT SCATIC NERVES IS BIOCHEMICALLY IDENTICAL TO TROPHIC ACTIVITY IN EMBRYONIC CHICK EYES. R. Nishi, T. Kikutani and F.P. Eckstein. Dept. of Cell Biology & Anatomy, Oregon Health Sci. Univ., Portland, OR 97201

Neurons in the chick ciliary ganglion undergo a marked period of cell death that is dependent upon the presence of target tissues in the eye. It has been postulated that this period of cell death is triggered by neurotrophic factors. Recently, the cDNAs coding for rat (Söckel et al, 1989; Nature 342: 920) and rabbit (Liu et al, 1989, Science 246: 1023) sciatric nerve ciliary neurotrophic factor (CNTF) were reported; however, because Northern blot analysis indicated that the mRNA coding for rat CNTF was not detected in developing tissue, the possibility that CNTF may be present only under "pathophysiological" conditions rather than during neuronal development. We have recently (Nishi, Aeppli) reported the >8000-fold purification of a growth promoting activity (GPA) from adult chick sciatic nerves that is 50% homologous to rat and rabbit CNTF, thus GPA may be the chicken form of CNTF or a related molecule. The exact nature of this activity is not known however whether this activity is biologically identical to a trophic activity previously reported in embryonic chick eyes we ran parallel purification of the eye and sciatic nerve material. The chromatographic retention times of the active material from both sources were identical through every step of the purification which included DEAE chromatography, gel filtration, chromatoaffinity, and two runs of reverse phase HPLC. In addition, SDS PAGE analysis of the active fractions of both preparations revealed co-migrating 21.5 KD bands that co-purified with the biological activity. Thus, embryonic chick eyes contain a considerable amount of trophic activity for CG neurons that is biochemically indistinguishable from that found in adult chicken sciatic nerves, supporting the notion that CNTF is the trophic factor for this molecule. Funded by NS25767 (RN), ALSA (RN), AG07424 (PFE), and March of Dimes (PFE).


It has been postulated that the survival and development of ciliary ganglion (CG) neurons is regulated by neurotrophic factors released by target cells in the eye. Previously, a growth-promoting activity (GPA) was identified in both chick eye extracts (Nishi & Berg, 1981), and we have recently reported the >8000-fold purification and characterization of GPA from chick sciatic nerves (Eckstein et al., 1990). Amino acid sequence analysis of a probe digested fragment of GPA shows a 57% homology with a carboxy terminal region of rabbit and rat sciatic nerve CNTF (Liu et al., 1989; Stoeckel et al., 1989). The biological activity of GPA and its homology to mammalian CNTF suggests that GPA is the chicken form of CNTF or a related factor. In order to test whether GPA is a trophic factor for CG neurons we are examining the developmental- and cell-specific expression of the GPA gene. Degenerate oligos were synthesized based on the CNTF amino acid sequence and used in a PCR reaction with CDNA synthesized from chick eye and sciatic nerve RNA. Both sources of RNA produced amplification products that hybridized at low stringencies to a rabbit CNTF probe. Thus, the RNA coding for GPA is found in the developmentally relevant source. We expect to use these amplification products as probes to begin analysis of the complete GPA CDNA cloning from a cDNA library. The PCR-derived probe will also be used concurrently to analyze GPA gene expression with northern blots. The sequence of the CDNA clone will allow comparison of eye and sciatic nerve mammalian CNTF. We are especially interested in whether chick eye GPA contains a consensus signal sequence that is lacking in the sciatic nerve GPA. Supported by NS25767 (RN), AG07424 (PFE), March of Dimes (PFE), and the ALS Association (RN).
647.9

We have investigated the relationship between salivary nerve growth factor (BDNF) and fibromyalgic pain. Chick dorsal root ganglia (DRG) contain two subpopulations of neurons, 40% depending on BDNF for survival and 40% depending on neurotrophin-3 for survival. The 1989 fibroblast-conditioned medium (LC) promotes the survival of 95% of DRG neurons, suggesting that it contains both BDNF and NGF. In the DRG, LVK 1-4, and LC also promotes neurite growth from retinal explants, which do not respond to BNGF, and L cells contain mRNA coding for BDNF. Antisera to BDNF and NGF are also present in the DRG, suggesting that NGF and BDNF have similar regions exposed to an aqueous environment and may share common antigenic sites. Antibodies to two synthetic peptides BNGF confirm that both NGF- and BDNF-like activities in LC CM arise from molecules that share at least one functionally important epitope. In addition, NGF-like molecules in LC CM can be distinguished immunologically from BDNF. Thus NGF- and BDNF-like molecules in LC CM may differ from BNGF and pig brain BDNF, and L cell-derived forms of NGF and BDNF are immunologically related.

647.11

The structural similarity between NGF and BDNF and their neurospecific activity suggest that a functional, functionally distinct members of this protein family may exist. Degenerate oligonucleotides, derived from the conserved sequences between NGF and BDNF were used as primers to amplify human placental DNA by polymerase chain reaction (PCR). Fragments of the expected size (500 bp) were identified and used as either NGF or BDNF-related sequence derived NT-3 clonings. The most complete NT-3 clone to date was isolated from a human fetal NT-3 cDNA expression library. A complete NT-3 sequence encoding 257 amino acids was obtained by screening a human genomic library with a complete BDNF 1.2 kb clone, encoding a 247 amino acid protein, which was obtained from a human hippocampal cDNA library. The nt-3 precursor protein is 44.2% and 38.5% similar to those of the NGF-like peptides and BDNF precursors, respectively, while the mature peptide forms shared 57.6% and 55.6% identity. The mature peptide forms of human and rat NT-3, and human and porcine BDNF, are identical. Northern blot analysis revealed a broad organ distribution of nt-3 mRNA including brain, liver, kidney, skin, spleen, lung, and in several brain regions. Rat BDNF mRNA was most prevalent in the brain, however, BDNF mRNA was detected in heart and lung suggesting a role in the peripheral nervous system. Recombinantly expressed and purified NT-3 was active in the survival of dispersed chick embryonic day 10 sympathetic ganglia (SG) neurons. Purified nt-3 had potent neurotropic activity in nodose ganglia (NG), having only 40-60% of dorsal root ganglia (DRG) NGF receptors survive relative to those in the presence of NGF. Thus, NGF and DRG neuronal systems, which innervate primarily visceral and somatic tissues, respectively, may respond to distinct trophic factors because of different target specificities.

648.1
LACK OF EFFECT OF CHRONIC ADMINISTRATION OF AGONISTS AND ANTAGONISTS ON RAT CNS RECEPTOR mRNA LEVELS. Z. Zang, M. Riva, H. H. M. Van Tol*, O. Crevillo* and Jan Croese. Center for Molecular & Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ 07102, *Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201.

Chronic treatment with an agonist or antagonist can change the density of CNS receptors. To gain some understanding of the molecular basis of the receptor regulation the present studies examined the mRNA levels for the muscarinic receptors (M1-4) in frontal cortex, amygdala, caudate, dopaminergic receptor (D2) in striatum, and the serotonergic receptor (5-HT2) in striatum and frontal cortex by using Northern blot analyses. Following two to three weeks treatment via implanted minipumps with scopolamine (0.5 mg/kg/day) a dopamine receptor antagonist, haloperidol treatment (0.5 mg/kg/day) a dopamine receptor antagonist, DOI (7.5 mg/kg/day) a serotonergic antagonist or ketanserin (7.5 mg/kg/day) a serotonergic antagonist was used. There were no differences between the control and experimental groups of mRNAs for M1-4, D2 or 5-HT2 receptors respectively in any brain regions, even though receptor binding showed that these chronic treatments had produced an increase or decrease in the total population of receptors. Within the sensitivity of the assays, these results suggest that the up-regulation of muscarinic receptors and the D2 dopaminergic receptor by antagonist treatment as well as the down-regulation of the 5-HT2 serotonin receptors by either agonist or antagonist treatments may not be directly regulated at the level of gene transcription.

648.2
PROTEIN SYNTHESIS IS REQUIRED FOR THE DENERVATION-INDUCED UP-REGULATION OF ACETYLCHOLINE RECEPTOR GENES. Hueh-Jen Tsay*, Craig M. Neveill, and Jakob Schmidt- Draxl. Department of Biochemistry and Cell Biology, State University of New York at Stony Brook, Stony Brook, New York, 11794.

Denervation of skeletal muscle stimulates transcription of acetylcholine receptor genes. In order to establish this activation is mediated by the appearance of an activator or the loss of an inhibitor we have investigated the effect of the protein synthesis blocker cycloheximide on the denervation response. White Leghorn chicks (1.1 g) were subjected to sciatic denervation or sham surgery of the sciatic nerve, and cycloheximide treatment (i.p. injections of 0.2 mg/kg at 4-hour intervals) was begun. 24 hours later the denervated muscle was assayed by probe excision solution hybridization for acetylcholine receptor a-subunit message and by transcription elongation analysis for a- subunit gene activity. In the presence of cycloheximide the increase in a-subunit message that normally follows denervation was not observed, and a-subunit gene activity dropped to about non-denervated control levels. Block of transcriptional activation was also seen in the case of the receptor b- and y-subunit genes. Several muscles tested were affected by cycloheximide, either in their steady-state levels or in the transcription rates of their mRNAs. During cycloheximide treatment of chronically denervated animals, a-subunit gene transcription was suppressed. These results suggest that the de novo synthesis of a transcriptional activator is required as a mediating event in the signalling pathway linking the plasma membrane and acetylcholine receptor gene expression.
468.3 

DESENSITIZATION OF MUSCARINIC RECEPTORS THAT INHIBIT ADENYLYL CYCLASE IN CEREBELLAR GRANULE CELLS INVOLVES HOMOLOGOUS LOSS OF MUSCARINIC m1 RECEPTIVE PROTEIN (66k Da) AND HOMOLOGOUS INCREASE IN m1 mRNA CONTENT. W.J. Wiksic, S. McLean, A. Dobrzenski and J. Mitchellet. PGIN and Anatomy, Cell Biology Dept., Georgetown University, Washington, D.C. 20007. 

In primary cultures of cerebellar granule cells, carbacah (100 μM) quantitatively desensitizes muscarinic receptors that inhibit adenylyl cyclase (MAC). However, muscarinic receptor(s) that comprises the MAC response has not been cloned and it may be presumed to arise from the MAC to be the muscarinic m1 subtype. Irreversible labeling of muscarinic receptors on membranes prepared from granule cells with N,N-diethylpropylamine mustard (PDAMC) and separation by SDS-PAGE shows proteins of 92 K Da and 66 K Da in molecular size. In membranes treated with carbacah (100 μM, 1 hr), [3H]-PDAMC binding to the MAC response is reduced. We also show that when desensitized with a 66 K Da protein, a homophilic m1-like protein (Laur, et al. J. Biol. Chem. 260, 371-377, 1985) was desensitized with MAC of two events, a short-term process (carbacah 100 μM, 1 hr) whereby the MAC response returns within one hour of receptor/ionophoresis (1 μM atrogin, 1 hr) and a long-term process (carbacah 100 μM, from 6 to 24 hrs) whereby the MAC response does not recover after 1 h of reinsertion. Muscarinic m1 and m2 receptor mRNA content was analyzed by Northern blot analysis using probes provided by Dr. T. Bonner, NIH. 

Comparisons between Northern blotting of radial glia or ventricle mRNA, that were extracted from cultures exposed to 100 μM carbacah for 1, 6, and 24 hrs, showed an increased m1 mRNA content at 6 hrs only and no change in m2 mRNA content. Thus, the MAC receptor is shown to be the m1 subtype and desensitization of MAC results in homologous changes in the 66 K Da protein and homologous increase in its mRNA content.

468.4 


The p4-adrenergic receptor (p4AR) gene is transcriptionally upregulated by forskolin or forskolin-stimulated cAMP levels. Previous studies show this autoregulation resides in the 5′-flanking region of the p4AR gene (RPS, 86: 239-245, 1989). A 34 bp sequence from the p4AR promoter (-70 to -37), containing the sequence GTACCTA, confers responsiveness to cAMP in either orientation to a thrombin-regulated protease and when transfected into rat C6 glioma or human JEG-3 choriocarcinoma cells. Specific mutations in this sequence completely abolished stimulation. Overexpression of the catalytic unit of protein kinase A fully restored the induction of CAT activity by forskolin.

A 43 kD transcription factor (CREB); cAMP response element binding protein (CREB) binds to cAMP responsive elements through binding to specific sequences. Purified CREB bound to the p4AR cAMP response element (CREB) in gel mobility shift assays with an affinity identical to that for the CRE from the human glycophorin α-subunit gene, and failed to bind to mutated elements.

These results describe an autoregulatory mechanism by which a receptor (p4AR) stimulatory for adenyl cyclase exerts positive feedback regulation on its own expression.

468.5 

THE ENDOGENOUS NEUROPEPTIDE PEC-60 REDUCES THE AFFINITY OF DOPAMINE D2 AGONIST BINDING SITES IN RAT NEOSTRIAL MEMBRANES. G. von Euler, S. Fryre*, V. Matti and K. Funke. Depts. of Histology and Neurobiology, and Biochemistry, Karolinska Institute, Box 6040, S-10401 Stockholm, Sweden. 

Recently, immunoreactivity towards a PEC-60 (peptide with N-terminal glutamic acid, C-terminal cysteine and a total of 60 residues) antiserum has been demonstrated in all catecholaminergic neurons in the rat brain including dopaminergic ventral terminals in the neostriatum. In order to investigate possible interactions with dopamine D2 receptors, the effects of PEC-60 were analysed in vitro and in vivo. 

PEC-60 was produced from rat neostriatal membranes with a maximal increase of 34±18 at 10-100 nM of PEC-60 (basal K+ values of 34±18 binding were 226±15 pM). The number of binding sites were not affected by PEC-60 at these concentrations. 

These results indicate the presence of functional PEC-60 receptors in the neostriatum that interacts with dopamine D2 receptors within the plasma membrane. The present findings may be of relevance for the understanding of D2 receptor regulation and of D2 related diseases such as schizophrenia.

468.6 

DIFFERENTIAL REGULATION OF [H]-(+)-3-PPP AND [H] DTG LABELLED SIGMA BINDING SITES IN GUINEA PIG BRAIN MEMBRANES BY SUBCHRONIC HALOPERIDOL ADMINISTRATION. W. Karbon and K. Napier, Nova Pharmaceutical Corporation, Baltimore, MD 21224-2708. 

A previous study (Napier, K. et al. Neuosci. Abstr. 1989, 1236) indicated that [H]-(+)-3-PPP and [H] DTG label pharmacologically distinct sigma binding sites in pig brain membranes, suggesting that these sites might be differentially regulated in vivo. To test this hypothesis, male Hartley guinea pigs were treated for 14 consecutive days with either vehicle or the sigma antagonist haloperidol (1 mg/kg, p.o.). Sacrificed 4 days after the final drug treatment, and brain membranes prepared and assayed for [H]-(+)-3-PPP and [H] DTG binding. Whereas haloperidol treatment caused a 20% decrease in [H]-(+)-3-PPP binding, only a 15% reduction in [H] DTG binding was observed. The decrease in [H]-(+)-3-PPP binding most likely reflects a Bmax change since the affinity of the remaining sites for (+)-3-PPP treatment. A significant reduction in [H]-(+)-3-PPP binding, but not [H] DTG binding, was seen in membranes prepared from guinea pigs sacrificed 28 days after the final drug treatment. It is suggested that administration of chlormazaine (30 mg/kg, p.o.) or clonazepam (20 mg/kg, p.o.) did not affect sigma binding sites. These results suggest that [H]-(+)-3-PPP and [H] DTG labelled binding sites can be differentially regulated in vivo by subchronic haloperidol treatment, an effect which may contribute to the therapeutic efficacy of haloperidol. Furthermore, the findings suggest that haloperidol may not function as a sigma "antagonist" in vitro, since receptor down regulation is most commonly observed in response to prolonged agonist exposure.

468.7 


The rat central nervous system has been mapped for Type II glucocorticoid receptor-like immunoreactivity using monoclonal antibodies. In non-adrenocorticalized rats, neuronal immunoreactivity is predominantly nuclear. In most neurons, adenohypophyseal aboeilohes nucleus immunoreactivity and reduces numbers of immunoreactive neurons, while treatment with glucocorticoids increases the intensity of nuclear immunoreactivity as well as numbers of immunoreactive neurons. 

Using BUR2, a monoclonal antibody against the rat liver glucocorticoid receptor we have localized a subgroup of neurons in Cal and CA2 of the hippocampus, caudate-putamen, globus pallidus and habenula which show a different pattern of immunoreactivity. Type II glucocorticoid receptor-like immunoreactivity was detected, while treatment with corticosterone or aldestosterone abolishes immunoreactivity. 

Thus, alterations in intracellular localization of type II glucocorticoid immunoreactivity following adrenal cortical steroid manipulations are dependent on the neuronal type in the brain. 

Supported by NIH grant NS24144.

Gerstmann-Sträussler-Scheinker (GSS) is an autosomal dominantly inherited human neurodegenerative disease that can be transmitted to non-human primates and rodents through intracerebral inoculation of brain homogenates from patients. Recent studies of GSS demonstrated that transgenic mice expressing the human mutation (His272Glu) containing a murine PrP gene were protected from the disease. In this study we examined whether this murine PrP gene was sufficient for protection in transgenic mice expressing a murine PrP gene at codon 101 (homologous to codon 102 in humans) was well until 33 weeks of age when it became lethargic, ataxic, rigid, and paraparetic, rapidly deteriorating over 3 days. The brain of founder Tg 174 showed diffuse spongiform changes and proteinase K-resistant protein reactive with PrP antiserum. Transmission studies of founder Tg 174 brain homogenate to mice are in progress. Two progeny of the Tg 174 founder are well at 19 weeks. Our results indicate that the clinical and pathological features of GSS may be reproduced in a Tg mouse paradigm. Successful horizontal transmission of disease from Tg 174 would impose new constraints upon models of prion structure.


During the course of making transgenic mice, we found an insertional mutant that expressed an abnormal circular behavior consistent with the PrP transgenic phenotype. Three transgenic mice were generated from a targeted knockout. One insertional mouse expressed the circling phenotype, while heterozygous mice did not. The inheritance of the phenotype was consistent with an autosomal recessive mode at a single locus. We found that the dopamine D2 receptor binding sites in pooled striata (both sides combined) of the circling mice were significantly elevated by about 13% compared to striata of normal heterozygous transgenic mice. We also found levels of several neurotransmitters (dopamine, serotonin, norepinephrine, and their metabolites (dihydroxyphenylacetic acid, homovanillic acid, 5-hydroxyindoleacetic acid) in the striata, nucleus accumbens, frontal cortex, and hypothalamus of mutants compared to heterozygous mice. We also did not find evidence of any hearing loss or inner ear deformities or degeneration in our insertional mutants. A 20% fragment from the host genome flanking the 3' end of the integrated transgene was found to map to mouse chromosome 16 by analysis of the strain distribution patterns of RFLPs in recombinant inbred strains. The transgene integration in the Tg153 mouse line disrupted an endogenous locus affecting motor function. It is the first instance in which insertional mutation has resulted in a well-characterized behavioral abnormality.

469.4 PROFILE OF MESOCORTICOLINE DOPAMINE NEURON LOSS IN HEATER MUTANT MICE DURING EXPER. B. Ghetti and L. C. Triarious. Department of Pathology (Neuropathology) & Program in Medical Neurobiology, Indiana University School of Medicine, Indianapolis, IN 46202.

Weanling male mice (C57BL/6J) live to 24-30 months on the B6;129-A/-2 hybrid stock; loss of midbrain dopamine (DA) neurons by FOG and FOG is part of their phenotype (Burger, Brain Res. 270: 256-263, 1988). In the present study we (1) investigated whether midbrain DA cell loss occurs in wild-type (+/+) B6;129-A/-2 mice with age, and (2) extended the analysis of DA cell loss to younger animals by obtaining 10-28 day old animals. Counts of Tyro3-like immunoreactive neurons did not reveal loss in any of the mesencephalic DA cell groups (SN, A8, A10) of +/- mice. The average number of neurons (+/+) mice of all ages (n=3) was 1270 ± 42 in 12 sets of SN and 3750 ± 126 in A10. Heated mutant mice were studied at 28 days (n=3), 12 (n=3), 18 (n=1), and 24 (n=3) months. During the first year of life, the maximum loss, amounting to 50% in A10, 70% in A8, and 51% in A10, was already observed at 3 months. At 2 years of age, losses are severe and amount to about 60% in A8, 80% in A8 and 56% in A10. These findings indicate that a second wave of DA cell loss in weaver mice, taking place in an older age but at a slower rate, is already present. This remains to be established whether such sensitive loss represents an accelerated form of aging of generally susceptible neurons or an effect of the mutation irrespective of aging phenomenon. (Supported by PHS ROI-NS44262).


Compared to normal (+/+) mice, the weaver mutant mouse (w/v/w) has increased dopaminergic input to the striatum from the substantia nigra (SN). In 5 pairs of 3 month old +/- and w/v/w mice, dopamine (DA) levels and tyrosine hydroxylase (TH) activity were determined by HPLC-EC, and DA uptake was measured in sucrose homogenates from the same striatal samples. DA levels and TH activity in the w/v/w mice were decreased approximately 60% relative to +/- controls. Striatal DA uptake in the w/v/w was decreased by 95%, thus being more severely affected than either DA content or enzyme activity. The present neurochemical data on DA content and TH activity are in agreement with previous morphological data on the w/v/w mouse. Since DA uptake is more severely affected than the aforementioned parameters, it appears that the remaining DA neurons in the w/v/w striatum are functionally inadequate.

Kinetic studies revealed that in other mice and indicated that the w/v/w enzyme had a higher Km and a lower Vmax than control. While neither the changes in Km nor Vmax alone seems to account for the observed reduction in TH activity in the w/v/w, such reductions may be attributed to the combined kinetic shifts. (ROI BS 144252)

469.6 ALTERED DEVELOPMENTAL AND TISSUE SPECIFIC REGULATION OF GENE EXPRESSION IN MOUSE TRISOMY 16, A MODEL OF DOWN SYNDROME. D. M. Haleman, R. M. Baynes*, C. N. Berger*, C. J. Eppig*, and W. C. Mobley. Deps. of Neurology, Pediatrics, and Biochemistry, UCSP, San Francisco, CA 94143; Molecular Therapeutics Inc., West Haven, CT 06516.

Our goal is to understand better how mammalian anoploidy affects gene expression. Mouse trisomy 16, an animal model of Down Syndrome, provides an opportunity to study the regulation of gene expression in cells with a 50% increase in normal gene dosage. We have examined expression of the gene encoding the amyloid precursor protein (APP), located on mouse chromosome 16 and human chromosome 21, in the brain and other organs of fetal Ts 16 mice and adult Ts HGN chimera. Although a 1.5 fold increase in APP levels per trisomic cell might be expected, there are examples in human Ts 21 and mouse Ts16 of greater increases in brain. APP has 4 RNA transcripts which code for proteins 770, 711, and 695 amino acids in length. The principal one is the precursor amyloid source of CNP 177 RNA. Such a precursor amyloid expression was increased by at least 2 fold in Ts 16 versus controls; however, the increases were both developmentally regulated and tissue dependent. APP 695 RNA was found in very low levels in normal fetal lung whereas this transcript was of greater or equal abundance than both other APP transcripts in Ts 16 fetal lung. All 3 APP transcripts were increased 2 fold in Ts 16 whole brain versus control control brain. The 695 mRNA in APP 770, which is 40-50% Ts 16, showed approximately a 5 fold increase in all APP transcripts versus controls. This increase is more than 50% of the dose and suggests more conserved as has been observed for other loci. Although the mechanism of this changes is unclear, our results with this gene suggest that an increase in gene dosage may initiate a complex change in the regulation of the affected gene. The notion that down syndrome is a single gene disorder is no longer tenable. We have developed a mouse model that can be used to test many of the hypotheses that have been developed in Down Syndrome. (Supported by PHS ROI-NS44262).
469.7

**Technetium Autoradiographic Analysis of Brain Lesions in T Cell Receptor Transgenic Autimmune Mice**


We have established that MRL-lpr/lpr (MRL-L) systemic lupus erythematosus (SLE) mice show multiple regions of decreased tracer uptake in autoradiographic sections of the central nervous system (CNS) on 99mTc-Hexamethylpropyleneamine Oxime (HMPAO) autoradiography which can be attributed to loss of blood brain barrier (BBB) integrity. These autoradiographic defects are not seen in MRL-lpr normal controls. The histopathologic nature of these defects are unlike other organ specific lesions in that they do not show characteristic perivascular cuffs, and thus may be mediated by amyotrophy production. To determine if auto-reactive T cells were involved, experiments utilizing MRL-L mice backcrossed to T cell receptor (TCR) transgenic (TR) mice were performed. In TCR-TR mice, most thymocytes are reactive with H-2D and the male H-Y antigen. The mouse brains were prepared for autoradiography after an I.V. injection of 4 mCi of 99mTc HMPAO in 0.1 ml of saline solution. These and other brains were then stained with hematoxylin and eosin. Serial 20 micron thick sections showed no identifiable characteristic defects in the TCR-TR MRL-L mice as was observed in non TRL MRL-L mice. Using the horseradish peroxidase (HRP) method to evaluate BBB integrity, there was no evidence of parenchymal infiltration of HRP as seen in MRL-L mice. In conclusion, the presence of the T cell receptor transgene eliminated the characteristic defects seen in MRL-L mice. This suggest that a normal T cell receptor repertoire is necessary for development of the lesions, but direct interaction of T cells with the brain might not be required. We propose a two step model for development of the CNS defects: 1) antigen specific T cell autoreactivity and endothelial antigens distant from but cross reactive with BBB endothelium and 2) the production of autoantibodies which cross react with BBB endothelium. This hypothesis suggests that use of medical therapy that enhances BBB integrity rather than those that suppress direct lymphocyte mediated tissue inflammation in the treatment of CNS disease of SLE.

469.9

**SYNAPTOSOME UPTAKE OF [3H]NOREPINEPHRINE, [3H]DOPAMINE AND [3H]SEROTONIN IN CANINE NARCOLEPSY**

J. McElwain, W. C. Dement and E. M. Mignot. Sleep Research Center, Stanford University School of Medicine, Palo Alto, CA 94304 (USA).

Canine narcolepsy, a model of the human REM sleep disorder, is associated with altered catecholamine metabolisms in various brain areas. A possible explanation for the sleep attacks could be the existence of specific defects in monoamine uptake processes. In order to investigate this hypothesis, we have studied the uptake of [3H]norepinephrine (NE), [3H]dopamine (DA) and [3H]serotonin (5-HT) by synaptosomes prepared from slowly frozen brain cortical tissues (Dodd, Neurochem Path 4: 177-178, 1986) in six narcoleptic (N) and six control (C) dogs. Neuronal (N) and cortical (C) control and narcoleptic dog cortical brain samples studied. No significant differences in Vmax or affinity (Km) for the transporter. All subsequent experiments were carried out using frozen Nauticals. Significant uptake processes for [3H]NE, [3H]DA and [3H]5-HT were found in all control and narcoleptic dog cortical brain samples studied. No significant differences in Vmax or Km were seen between N and C control animals. Noticeably, [3H]Norepinephrine Vmax and Km were found to be higher in both fast and slow samples of the control dogs. Further analyses will be studied to confirm this observation. The same protocol will be applied for the amygdala, a structure a generalized abnormalities have been well characterized in narcoleptic dogs. Research supported by NS 23724-03.

469.11

**GENES THAT CAN MUTATE TO INDUCE NEURONAL DEGENERATION IN C. ELEGANS.**

M. Driscoll* and M. Chalfie.

The molecular analysis of genes encoding touch receptor neurons in Caenorhabditis elegans has revealed that dominant alleles of six genes, mec-4 and deg-1, produce abnormal, toxic products that result in the vacuolar degeneration and death of small groups of neurons in the nematode. M. Driscoll* and M. Chalfie.

**COMPLEX NEURAL SYSTEMS AND BEHAVIORS: A NOVEL STRATEGY FOR THEIR GENETIC DISSECTION.**


Most aspects of brain function are complex and genetically variable. To eliminate major obstacles in the genetic analysis of catecholamine neurotransmitter mechanisms, we initiated a study to transfer genes that influence the activity of mesencephalic tyrosine hydroxylase (TH/MEIS), the rate limiting enzyme in catecholamine biosynthesis, to the same genetic background. The transfer of genes was carried out by backcross-intercross cycles with concomitant selection for TH/MEIS. We have successfully completed five cycles establishing replicated high (B6.C alpha and beta) and low (B6.I alpha and beta) genetically standardized stocks. The B6.C and B6.I populations evince a highly significant difference in TH/MEIS, while at cycle 4, the probability of increases at any nonlinked, nonselected locus is 0.948. Development of sets of congenic recombinant inbred, neurologically normal strain lines with different mesencephalic dopamine systems provides an analytical tool for mechanism-oriented experimentation.

Different oligonucleotides from the coding region of the rat 5-HT[subscript 4] receptor gene were used to examine the localization of transcripts for this receptor in the rat brain by using in situ hybridization histochemistry. The specificity of the hybridization signals was verified in control experiments and Northern analysis. The results obtained indicated that the presence of transcripts was abundant in the hippocampus, lower in the midbrain and cortex, and absent in the cerebellum. The highest levels of hybridization signal were seen in the hippocampus, entothral cortex, septum, nucleus of the vertical limb of the diagonal band, interpeduncular nucleus, all raphe nuclei, ventral nuclei of the lateral lemniscus, olfactory bulb and cerebral cortex. This distribution was in very good agreement with that of receptor binding sites labeled with [3H]DPAT. These results demonstrate that different cerebral regions express the 5HT[subscript 4] receptor mRNA. They also suggest that the same gene codes for presynaptic autoreceptors, postsynaptic autoreceptors, and postsynaptic receptors in the cholinergic cells of the septum and postsynaptic receptors in the hippocampus.


We have assayed the heave of [3H]haloperidol (HAL) for imaging and quantifying brain 5-HT receptor sites in vivo. HAL uptake in striatum (ST), cerebellum (CB), and cortex (Cx) peaked at 30 min post-injection in naive C57Bl/6J male mice. 


Based on binding studies in various tissues and species, evidence for several alpha-2 adrenergic receptor subtypes has accumulated. Currently the alpha-2 receptors are classified exclusively by pharmacological criteria. The molecular cloning of three distinct genes for human alpha-2 adrenergic receptors confirmed the existence of at least three receptor subtypes. The distribution of these receptor subtypes in rat tissues was determined by using techniques which were shown to be valid as demonstrated by simultaneously measuring the calcium responses to the agonist phenylephrine, which stimulates all three receptor subtypes. The three receptor subtypes exhibited distinct pharmacological profiles and were associated with different developmental stages and tissue specific gene expression. These results confirm the presence of multiple alpha-2 receptors in rat tissues and suggest that these receptors play distinct roles in different physiological processes.

470.4  AUTORADIOGRAPHIC LOCALIZATION OF PUTATIVE SIGMA RECEPTORS IN THE PRIMATE AND HUMAN BRAIN. A.E. Carlberg and D. C. Mash. Departments of Neurology and Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

Sigma receptors are the current target for antipsychotic drug development. Novel antipsychotic agents which possess selective and high affinity for the putative sigma binding site have demonstrated beneficial effects in schizophrenic patients in recent clinical trials. These agents may serve as an alternative to the principal neuroleptic drugs currently in clinical use which mediate extrapyramidal side effects and dyskinesias through their blockade of dopamine receptors. We have used in vivo autoradiography to localize putative sigma receptors labeled with (+)-[3H]3-(3-hydroxyphenyl)-N-(1-propyl)piperidine (K(+)-3-P) in the primate and human brain. The binding characteristics of K(+)-3-P in the primate brain were comparable to those previously described in the rodent. Saturation analysis demonstrated a single class of sites in cerebral and basal ganglia (Kd = 100 ± 20 nM). Computer-assisted densitometry demonstrated that all paralimbic and limbic regions including the amygdala, hippocampus, orbitofrontal, cingulate, insular, and temporal areas displayed peak densities of K(+)-3-P binding. Moderate labeling of sigma receptors was seen throughout the hypothalamus. A striking enrichment of binding was apparent over the supracallosal and suprapagosal nuclei. Moderate to low levels of sigma binding sites were observed over the ventromedial sectors of the caudate and the putamen. Within the brainstem, sigma receptors were elevated over the cerebellar vermis. Taken together, these observations suggest an association of sigma receptors with the limbic system. The targeting of novel sigma-selective agents to limbic brain areas may explain, in part, the beneficial effects of these drugs in schizophrenia. Supported by DA0227.

470.5  PARTIAL OVERLAP IN THE DISTRIBUTION OF MONOAMINO oxidASE TYPE A (MAO-A) AND SIGMA RECEPTORS IN RAT AND MOUSE BRAIN. M. Basle, D. C. Mash, and Y. Jirahk. Dept. of Neurology, Pharmacology, Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL 33101.

Biogenic amines are metabolized by monoamine oxidase. Selective inhibitors of MAO type A (i.e., tranylcypromine) exhibit high affinity for sigma binding sites (Eur. J. Pharmacol. 176: 107, 1990). The present study was undertaken to examine the distribution patterns for [3H]MPTP binding to MAO-A and [3H]3-P binding to putative sigma receptors. MAO-A sites were labeled in rat and mouse brain with [3H]MPTP in the monkey. By using techniques which were shown to be valid as demonstrated by simultaneously measuring the calcium responses to the agonist phenylephrine, which stimulates all three receptor subtypes. The three receptor subtypes exhibited distinct pharmacological profiles and were associated with different developmental stages and tissue specific gene expression. These results confirm the presence of multiple alpha-2 receptors in rat tissues and suggest that these receptors play distinct roles in different physiological processes.

470.6  MUSCARINIC BINDING SITES IN THE DORSAL MEDIAL MEDULLA: AUTORADIOGRAPHIC AND BIOCHEMICAL STUDIES. E. Frigerio, Dept. of Medicine & Neuroscience, Case Western Reserve Univ., School of Medicine, Cleveland, OH 44106.

Structures in the dorsal medullar (DMMD) mediate cardiorespiratory control. Muscarinic receptors participate in central autonomic regulation. In this study, muscarinic receptors labeled with [3H]quinuclidinyl benzilate (QNB) were characterized in rat brain sections and cow DMMD membranes. Quantitative image analysis of autoradiograms from rat medulla sections (15 μm) labeled with [3H]QNB (1 nM) in Krebs, 60 min at 4°C revealed that muscarinic binding (fmol/mg tissue, mean±SE of 15-66 determinations) was heterogeneously distributed in the hypoglossal complex (m 316±8, n 267±4, d 259±4, Raterer 33±1, NTS 13±63, vl 125±90), e 142±8, dm 209±6, d 140±3, n 122±4, n 149±3, Ap 52±3, C2 area 147±6, n parahippocampi 89±3). Outside the DMMD, muscarinic binding was abundant in the bed nucleus of the stria terminalis, the substantia nigra and the parapyramidal area (542±11). Membrane binding assays with cow DMMD showed a high density (B(max) 31±10 fmol/mg protein) of high-affinity (K(d) = 0.450±0.52 μM) [3H]QNB binding sites. Muscarinic binding sites competed for [3H]QNB binding with the following order of potency (Ki in μM): scopolamine (1.63±3) > D-AMP (2.65±3) > atropine (3.16±7) > piniprerin (10.62±16) > methacholine (10.78±23) by 74% in the M3 subtype. Hill slopes were close to one, indicating a single population of sites. For agonists, the order of potency was oxotremorine (102±8) > pilocarpine (110±120) > methacholine (801±830) > N,N,N′,N′-4-[(dimethylamino)carbonyl]-4-chloroaniline (343±(60000±700), also consistent with the M3 subtype. In DMMD, muscarinic binding sites are present in structures with cardiorespiratory function and appear to be exclusively of the M3 subtype.

The phosphostide (PI) second messenger system mediates numerous neurotransmitter effects in the brain, which, with some exceptions, have not been readily assignable to specific cellular sites. Localization of neurotransmitter synaptic responses in the brain has been explored by autoradiographic mapping of receptor binding sites, but these sites sometimes do not reflect known synaptic specificity. We would like to image functional, second messenger responses to neurotransmitters at specific loci in the brain. However, monitoring the generation of [3H]inositol phosphates in response to neurotransmitter agonists is not compatible with anatomical localization. Recently, Goffrey (Biolchim, 1(1989) 258-621) measured PI turnover in brain slices with [3H]lysidine as a precursor. In this technique we generation of [3H]lysidine diphasically and diacylglycerol ([3H]DAG)-DAG) reflects PI turnover. Since CDP-DAG is membrane bound, we attempted to localize [3H]DAG-DAG by autoradiography, rinsing away water soluble metabolites. Using [3H]lysidine as a precursor, we report discrete localizations of phosphostide turnover in brain slices and peripheral tissue by selective autoradiography of [3H]DAG-DAG.

471.8 AUTORADIOGRAPHIC LOCALIZATION OF FLUNITRAZEPAM BINDING SITES IN DIAZEPAM-SENSITIVE AND -RESISTANT MICE. E.J. Gathah, S.E. Doner*, J.K. Belknap, VA Medical Center and Dept. of Pharmacology and Medical Psychology, Oregon Health Sciences Univ., Portland, OR 97201.

Diazepam-sensitive (DS) and -resistant (DR) mice were developed by selective breeding based on the duration of a footrot, following a standard dose of diazepam. Initials of 3H-flunitrazepam (FLU) binding failed to indicate differences between DS and DR mice in either whole brain or in dissected brain areas (parahippocampus, cortex, amygdala). However, significant differences in small anatomical areas would not be observed using this method. We therefore initiated an autoradiographic study to survey the density and location of various [3H]-flunitrazepam receptor ligands throughout the mouse brain. In the current study we report the distribution of [3H]-FLU binding throughout the brain of DS and DR mice.

Brains were sliced into 16-micron sagittal frozen slices, thaw-mounted on slides, and stored frozen until incubation with ligand. Slices from paired DS and DR mice were incubated simultaneously and were then exposed to [3H]-sensitive film for seven days. Images were photographed with a video camera, digitized, stored on a hard disk drive, and analyzed with a MicroComp DS microdensitometry system. Brain areas were delineated and receptor densities were determined after subtraction of background density. We quantitated receptor density in whole brain, cerebellum, cortex, hypothalamus, thalamus, corpus striatum, and inferior colliculus, and substantia nigra. Our initial analyses suggest that differences between DS and DR mice are either small or insignificant, consistent with the earlier studies. These findings indicate that behavioral differences observed in DS and DR mice are not a reflection of receptor densities and that DS/DR behavior is a result of alterations in receptor subunit structure and function.

Supported by PHS Grant NS29397 and the VA Medical Research Service.

471.1 EFFECTS OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS (CEI) ON BRAIN ANGIOTENSIN II (AII) BINDING. K.L. Bercoek and B.H. Swords* Hypertension Res., Univ of Alabama, Birmingham, AL 35294

Brain and neural tissues from SHR have an increased number of AII receptors in comparison to controls. In order to determine whether there was an alteration in AII binding in SHR after CEI, we compared AII binding in neuron-enriched primary cultures of whole brains from 1 day old SHR pups treated in utero with Captopril (CP) or control SHR (CON) pups and studied the effect of short term incubation of neural cells from CON and CAP SHR and nonneuronal rats (NR) with CAP or lisinopril (LIS). AII binding in neural cultures from CAP SHR was decreased when compared to cells from CON SHR. Scatchard analysis revealed no differences in Kd but Bmax was less in CAP SHR (7.5) than in CON SHR (9.3fmol/mg protein). Short term (24 h) incubation of cells with CAP or LIS (10^-6M) did not affect AII binding in neuronal cultures from NR. All AII binding was decreased in SHR cells treated with CAP (CON SHR: Kd = 0.82 nM, Bmax 9.3 fmol/mg protein vs CON SHR + CAP: 0.5, 7.5) moreover, a significant increase occurred by 3 days in control SHR. All AII binding was also decreased in SHR treated with CET (CAP SHR: 0.32, 7, 1; CAP SHR + CAP 0.29, 6, 1) but to a lesser extent than CON. These data suggest that CEI decrease AII binding in brain from SHR by competitive inhibition or receptor internalization and the AII receptor in SHR may be regulated differently from that of NR.


Recent studies with new, selective angiotensin II (AII) antagonists have revealed AII receptor subtypes (Gallagher, Biochem. Pharmacol. 42, 651–659, 1990). Since these receptors can be discriminated by their binding sensitivity to sulfhydryl reducing agents, and our studies indicate that central AII receptors are differentially sensitive to loss of the sulfhydryl (SH) group (M) in the rat (Gage, J. Neurosci. 4, 166, 1984), we concluded that central AII receptors occur as different subtypes also. We conducted competitive autoradiography with the nonpeptidic AII receptor subtype selective antagonist, DuP733 (gift from DuPont). Rat brain sections were incubated with 240-270pmol [3H]AII (all-site [3H]AII) alone, or with AII (10^-6M) or with captopril (CP) (10^-6M) alone incubation was displaced by 10^-6M DuP733 (designated AII receptor subtype) at some sites, but was unaffected by 10^-6M DuP733 at other sites (AII receptor subtype). Based upon the selective competitive displacement of DuP733 at different subtypes was concluded to be an indirect activation of AII receptor subtype following exposure to AII. AII receptor subtype was categorized as having predominantly AII or AII subtypes as follows: AII: solitary trans nuclear (n), parahippocampal a, anterior piriformis p, and periventricular hypothalamic, or subarachnoid portion of the corpus callosum. AII: inferior olivary, kocher complex, and inferior colliculus, mostly thalamic areas and medial amygdala. [3H]AII binding is inhibited by M at AII sites but not at AII sites. Supported by Amer. Heart Assoc., Tenn. Affiliate and NIH (NS24388).
ANGIOTENSIN III AND P-AMINOPHENYLALANINE

Competitive binding studies with selective antagonists and differential effects of sulfhydryl reducing agents indicate specific sites of angiotensin II (AlI) receptors: a DuP 753-sensitive site (AlI.) and a DuP 753-insensitive site (AlI). The binding for the competitive binding of 125I-angiotensine I, isoleucine II (125I-Al, 300 pm) to 20 to 30 muh rat brain sections by the selective AIII (AlI., 100 pm) and p-aminophenylalanine AlI (pGlu-Fc, AlI, 1 pm) were compared to that for the heptapeptide, angiotensin III (AlI, 100 pm) and p-aminophenylalanine AlI (pGlu-Fc, AlI, 1 pm) using quantitative densitometry of autoradiograms. AlI and pGlu-Fc AlI displaced 125I-AlI binding in brain regions where AlI receptors predominated, e.g., septum, medial amygdala, thalamus, subthalamus, colliculi, locus coeruleus and inferior olivary, but it was a poor competitor for 125I-AlI binding in brain regions where AlI receptors predominated, e.g., circumventricular organs, median preoptic nucleus, paraffin cortex, solid tract nucleus, optic motor nucleus, spinal trigeminal tract. This indicates that agonists, including the endogenous peptide AIII, can distinguish brain region-specific AII receptor subtypes. Supported by NIH (NS24388) and Am. Heart Asn., TN affiliate. DuP 753 was a gift from Dr. Pieter Timmermans (Dupont).


Our previous studies demonstrated that peripheral-type benzodiazepine receptors (BPR) regulate steroid synthesis by mediating cholesterol transport within mitochondria. In these studies we have used the ACTH-stimulating response of MA-10 Leydig cells to examine the relationship between BPR and luteinizing hormone-stimulated steroidogenesis. Most PBR ligands tested had no effect on steroidogenesis by ACTH or hCG, however, flunitrazepam, at submicromolar concentrations, inhibited testosterone production induced by these hormones. BPR ligands 1-1 and MA-10 cells, respectively, or by 1 mM dibutyl cyclic AMP. This inhibition by flunitrazepam was characterized by a decrease in the efficiencies but not in the potency of the hormones stimulating steroidogenesis. This benzodiazepine affected a step following activation of the hormone receptors.

These findings support the possibility that hormone-stimulated steroidogenesis includes a mechanism involving the direct participation of PBR, for which flunitrazepam, a low intrinsic activity agonist, may act as an antagonist.


Our previous studies demonstrated that peripheral-type benzodiazepine receptors (BPR) regulate steroid synthesis by mediating cholesterol transport within mitochondria. In these studies we have used the ACTH-stimulating response of MA-10 Leydig cells to examine the relationship between BPR and luteinizing hormone-stimulated steroidogenesis. Most PBR ligands tested had no effect on steroidogenesis by ACTH or hCG, however, flunitrazepam, at submicromolar concentrations, inhibited testosterone production induced by these hormones. BPR ligands 1-1 and MA-10 cells, respectively, or by 1 mM dibutyl cyclic AMP. This inhibition by flunitrazepam was characterized by a decrease in the efficiencies but not in the potency of the hormones stimulating steroidogenesis. This benzodiazepine affected a step following activation of the hormone receptors.

These findings support the possibility that hormone-stimulated steroidogenesis includes a mechanism involving the direct participation of PBR, for which flunitrazepam, a low intrinsic activity agonist, may act as an antagonist.


Our previous studies demonstrated that peripheral-type benzodiazepine receptors (BPR) regulate steroid synthesis by mediating cholesterol transport within mitochondria. In these studies we have used the ACTH-stimulating response of MA-10 Leydig cells to examine the relationship between BPR and luteinizing hormone-stimulated steroidogenesis. Most PBR ligands tested had no effect on steroidogenesis by ACTH or hCG, however, flunitrazepam, at submicromolar concentrations, inhibited testosterone production induced by these hormones. BPR ligands 1-1 and MA-10 cells, respectively, or by 1 mM dibutyl cyclic AMP. This inhibition by flunitrazepam was characterized by a decrease in the efficiencies but not in the potency of the hormones stimulating steroidogenesis. This benzodiazepine affected a step following activation of the hormone receptors.

These findings support the possibility that hormone-stimulated steroidogenesis includes a mechanism involving the direct participation of PBR, for which flunitrazepam, a low intrinsic activity agonist, may act as an antagonist.
61.8

**SURVIVAL OF VASOPRESSIN (APV) AND OXYTOCIN (OT) NEURONS IS IMPAIRED BY HYPONATREMIA IN RATS WITH PITUITARY STALK INJURY.** Juan Debonis, G. E. Hoffman, and J. G. Verhasselt. Departments of Medicine & Physiology, University of Pittsburgh, Pittsburgh, PA 15261.

We have recently reported that compression of the pituitary stalk (SC) results in a biphasic neuronal death with approximately 70% of APV and 30% of OT neurons dying in the supraoptic (SON) and paraventricular nuclei paranucleus (PN). Other studies have shown that loss of APV but not OT neurons following stereotoxicity is not dependent on vasopressin (V1a) receptors. However, since DDVP has not been tested for metabolic activity in magnocellular neurons, in this study we determined the degree of chronically altered metabolism on the survival of magnocellular neurons following supraoptic (CH) compression. Because such an experiment has been conducted in animal models with increased hypothalamic osmolality, we have extended this study to the examination of whether vasopressin gene expression was altered in this situation. Thus, we examined the effects of either APV or OT infusions on rats with N-acetyl-D-arginine (DAAVP) on the survival of SON and paraventricular neurons in the SON.

61.9

**CATHECOLAMINE DEPLETIONS OF THE DIAGONAL BAND OF BROCA (DBB) ATTENUATE BARORECEPTOR SENSITIVITY OF RAT SUPEROPTIC (SON) VASOPRESSIN (AVP) NEURONS.** L.G. Grifeth and M.P. Renaud, Neuroscience Unit, Ottawa Civic Hospital, Ottawa, Canada K1Y 4E9.

In the rat, stimulation of peripheral baroreceptors by acute blood pressure increases in blood pressure will transiently inhibit vasopressin-secreting neurones. Previous research indicates this is mediated via the DBB. The present study evaluated the importance of the catecholaminergic innervation of the DBB in the baroreceptor sensitivity of vasopressin neurones in the SON. Adult male Long Evans rats were studied in combination with sodium nitroprusside in the DBB with 6-OHDA (4 µg/µl). Controls were injected with either vehicle (0.1% acetic acid in 0.9% NaCl) or the same dose of 6-OHDA after administration of the dopamine uptake inhibitor desipramine (5 mg/kg). Two weeks later, the rats were reanesthetized with nitrous oxide and prepared for extracellular recording of identified neurones in the SON using a transphenoidal approach. In vehicle and DMI control experiments, both phasic and tonic vasopressin neurones were inhibited by increases in blood pressure produced by metaraminol (10 µg/kg iv). Equimolar metaramin-induced pressure responses inhibited less than half of the phasic vasopressin neurones (8/18) in rats with 6-OHDA injected into the DBB. This decrease in baroreceptor sensitivity in the rats injected with 6-OHDA was significantly different from vehicle injected controls (X2(1)=4.07, p<0.05) and from DMI perfused rats (X2(1)=6.64, p<0.01). This study suggests that the noradrenergic innervation of the DBB is involved in the baroreceptor inhibition of vasopressin neurones in the SON. (Supported by NSR M60977, FRQS and the MRC of Canada.)

**PAIN: PATHWAYS II**

647.2

**SIGNALLING OF A STEP-LIKE CHANGE IN INTENSITY OF A NOXIOUS MECHANICAL STIMULUS: SEPARATING "SENSORY" FROM "AUTOMATIC" RAT SPINAL DORSAL HORN NEURONES Jennifer M.A. Laidl and Fernando Cervero, Dept of Physiological, University of Bristol Medical School, Bristol, BS8 1TD, U.K.

A noxious stimulus evokes a variety of responses; sensory (pain), motor (withdrawal, reflex), and autonomic responses (vasoconstriction). Peripheral nociceptors provide the input to all of these systems. The first possible point of division is the dorsal horn of the spinal cord. However, while dorsal horn cells participate in the different response, the division is not clear. A recent study of the dorsal horn, this problem was suggested by the results of experiments in which human subjects rated the intensity of the pain sensation produced by a noxious mechanical pinch applied to an interdigital web. The 120s stimulus had a step-like increase or decrease in intensity after 60s. Subjects detected a step increase but rarely a step decrease. The response latency varied from 150 to 400 ms, and 10% of the responses were also measured and found to follow both the step increase and the step decrease. We have now investigated the ability of neurones in the dorsal spinal horn of the rat to signal step changes in 120s pinch stimulus. Four pinches, 2 with a step increase and 2 with a step decrease in intensity (4N-6N, 8N-8N, 8N-8N, and 8N-4N), were delivered in random order to the medial, lateral, and ventral receptive fields on the tail. Cells with a noceptive input, either exclusively (class 3) or in addition to a less specific or a less noceptive (class 4) were recorded. 120 pinches. The 26 cells could be divided into 4 groups depending upon their responses to the pinch stimulus as follows: i) signalling both a step up and a step down (n=13) (fig. 1); ii) signalling either a step up or step down (n=4) signalling neither (n=2). Each of these groups contained both class 2 and class 3 cells, located throughout the dorsal horn. These results show that there are groups of dorsal horn neurones that follow the autonomic and the sensory output patterns. We conclude that different sub-groups of class 2 and 3 neurones appear to be involved in both sensory and in autonomic aspects of nociception.
472.3 LAMINA I TRIGEMINOThALAMIC PROJECTIONS IN THE MONKEY. A.B. Craig, Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.
Thermoreceptive and nociceptive lamina I neurons constitute half of the spinal input to the thalamus, and their termination sites signify important local form, temperature and pain sensation. Lamina I projections from the trigeminal dorsal horn have been identified in the cynomolgus monkey by using the PHAL anterograde tracing method previously employed successfully in the cat. Dense terminal clusters bearing large boutons are concentrated at the ventrocaudal (probable nociceptive region) and dorsomedial (possible thermoreceptive region) aspects of VPM. Strong mediodorsal thalamic terminations in the ventral aspect of caudal MD (adjacent to PF), which could be homologous to the submedial projection in the cat, are similar to the "paralaminar MD" projection described by Ganchrow (JCN 178:281, 1978) in the squirrel monkey. These findings offer unambiguous, substantive evidence for the location of clinically critical thalamic sites for trigeminal pain sensation. Supported by NS25616 and the Barrow Neurological Foundation.

5-HT receptors are located on vagal afferents; activation of these receptors produces a Bezold-Jarisch-like response. The purpose of this study was to determine whether phenylbiguanide (PBG), a 5-HT agonist, could influence spinal afferents by activating cardiac sympathetic afferents. Nine cats and 10 monkeys were anesthetized with chloralose and/or pentobarbital. Electrophysiological techniques were used to record activity of thoracic spinointracardial (N=17, STT: 8, SRT: 9) and non-projecting (N=4) neurons. PBG was injected into the left atrium using doses that had little or no effect on neuronal activity when injected into the ascending aorta; the most effective doses were 30-40 mg/kg. In response to PBG, 17 neurons were excited, inhibited, or had no response while 10 neurons were inhibited. The observation that these responses were not post- and/or post-vagotomy. Three of these cells were excited by PBG, and the response was comparable or bigger after vagotomy. The other cell was inhibited, and the inhibition blocked by vagotomy. In 10 neurons responsive to PBG and assessed for responses to left vagal afferent stimulation, the vagal response was in a different direction than the PBG response. Taken together, the results suggest that thoracic STT and SRT cells are influenced by 5-HT, and that this sympathetic afferents. (Supported by NIH grants HL22732 and HL26018.)

472.5 SPINAL NEURONS WITH BRANCHED AXONS PROJECTING TO THE SOLITARY TRACT AND DORSAL COLUMN NUCLEI. K. Takao, Y. Iwamoto, I. Yamakou. Dept. of Neurobiology, College of Medicine, University of Tokyo, Tokyo, Japan.
Retrograde labelling and antidromic action potential techniques were used to identify the dual projection of spinal neurons to both the solitary tract nucleus(STN) and dorsal column nucleus(DCN). 
Experiments were conducted on adult rats anesthetized with sodium-pentobarbital. Propidium iodide(Pi) and Bishenzyline (BB) were injected into the STN and DCN respectively in 10 rats. Antidromic stimulation was alternatively delivered to STN and DCN in another group of 26 rats. The distribution of Pi and BB labeled cells and STN and DCN activated neurons were respectively identified in the lumbar dorsal horn.
Two hundred and eighty two cells were found to be labeled by Pi and/or BB in the lamina III-VI. Eighteen percent of the sample (51 cells) were found to be labeled by both Pi and BB. A total of 52 neurons in laminae III-VI were found to be driven by stimulation of the STN and/or DCN. Of them, 38 and 54 neurons were found antidromically and synthetically to respond to both the STN and DCN stimulation. Synaptic responses were also shown in 6 of the 38 neurons that responded antidromically to both the STN and DCN.
These results indicate that some spinal neurons issue branched axons projecting to both the STN and DCN and some of these dual projection neurons are innervated in turn by the STN and/or DCN. In addition, some spinal neurons are doubly innervated from both the STN and DCN.

472.7 PROJECTIONS TO THE VENTRAL PERIPHERY OF THE CAT'S THALAMIC VENTRAL POSTEROMEDIAL NUCLEUS (VPM). C. Vahle-Hinz and K.-D. Kuffki. Physiologisches Institut, Universität Würzburg, D-8700 Würzburg, FRG.
The VPM is a thalamic nucleus in the nociceptive system of the cat. Neuronal tracers (HRG, WGA-HRP, WGA-HRP-gold) were injected into this region to retrogradely label the neurons of origin of its inputs from brainstem, thalamus and cortex. All tracers used produced similar results, however, the number of labeled neurons was consistently higher with WGA-HRP-gold. Retrogradely labeled neurons were found contralateral to the injection site in the spinal trigeminal complex, especially in the marginal lamina of nucleus caudalis, in the principal and geminal trigeminal nuclei and in the periaqueductal grey. Ipsilateral to the injection neurons were labeled in the dorsal principal trigeminal nucleus, the parabrachial complex, the periaqueductal grey, the thalamic reticular nucleus and the cingulate cortex. Since the VM is small and several pathways traverse this region, some spread of the tracer to VPM proper and retrograde labeling via damaged fibers of passage have to be taken into account. However, the responsiveness of neurons in the VPM to mechanical, thermal and chemical stimulation of visceral organs and baro- and chemoreceptors suggests that fibers from the spinal trigeminal and parabrachial nuclei may be these inputs and terminate within VPM. In addition, neurons from the cingulate cortex, the thalamic reticular nucleus and the periaqueductal grey may modulate excitatory influences. Supported by the Deutsche Forschungsgemeinschaft.

The superior sagittal sinus (SSS), as well as other cranial vessels and meningeal vessels are known to be pain sensitive in man. Studies of the central processing of pain from intracranial structures have focused on the first order neurons of the trigeminal system that synapse in the trigeminal nuclei. The electrical stimulation of the SSS causes metabolic activation of the trigeminal system which may explain the sensation of the head. The SSS was electrically stimulated in the chloralose-anesthetized (60mg/kg,ip), paralyzed and ventilated cat. Metabolic activity in this area was determined using the 2-deoxyglucose method with quantitative tissue autoradiography. In a group of animals metabolic activity was measured after bilateral trigeminal ganglion ablation. Stimulation of the SSS in the intact cats increased glucose utilization in the ventrobasal complex of the thalamus by 160% while no change was seen in surrounding structures. In the ablated cats stimulation of the SSS decreased glucose uptake by 50% while no change was seen in surrounding structures. No glial response was observed. These data are the first demonstration of craniovascular processing in second order neurons in the trigeminooccipital system. Furthermore these data clearly implicate the ventrobasal complex in processing craniovascular nociception.
472.1

NOCTURIA AND NOCICEPTIVE MODULATION OF TASK-RELATED RESPONSES IN AREA 7B CORTEX OF MONKEYS. W.K. Dong and Y.J. Huang, Department of Anesthesiology and Multidisciplinary Pain Center, University of Washington School of Medicine, Seattle, WA 98195.

Three distinct functional groups of neurons with somesthetic responses have been identified in the inferior parietal lobule (area 7b) of monkeys that performed a sensitive tolerance-escape task. Monkeys were allowed to initiate and terminate thermal stimulation of the face and were reinforced for completing trials that sometimes required tolerance of noxious temperatures. A nociceptive group of neurons had peak thermal frequencies that increased monotonically in response to graded thermal pulses. Maximal discharge frequencies were at temperatures near the pain tolerance level (+50°C escape). A task-related group of neurons displayed inhibition of background activity during the sensitization period following noxious stimulation. A reversal of this inhibitory period by presentation of tolerable warming pulses was seen in some task-related neurons, but optimal reversal of inhibition required intolerable warming pulses in all other task-related neurons. A third group of neurons had both trigeminal somatosensory and viscerosensory properties; their axonal discharge was elicited along common spatial coordinates. Slowly by a visual stimulus approach and staying near a face cutaneous receptive field. These groups may be part of a polysemy neural assembly that processes information used to guide the body away from stimuli that decrease tissue injury.

Supported by NIH grants NS07110, DE07617 and NS07217.

472.3


We have previously shown that 5-MT has a biphasic effect on development of serotonergic neurons in culture and the present study was carried out to determine if this was also observed in vivo. Pregnant Sprague-Dawley rats were injected s.c. with 0.1, 1 or 3 mg/kg 5-MT from gestational day 12 through birth. Terminal density was determined at postnatal day (PD) 1, 15 & 30 by measuring uptake of [3H]-5-HT. Serotonergic behaviors were tested at PD 5, 15 & 30. The lowest dose of 5-MT produced no change in terminal density at any timepoint but did produce significant behavioral changes at PD 30. The medium dose produced both a decrease in terminal density and behavioral changes at all timepoints. The highest dose produced an increase in terminal density at all timepoints but a decrease in behavioral alterations observed at early timepoints. These results confirm our findings on development of terminals and also suggest a role for serotonin in a receptor programming and subsequent serotonin behavioral sensitivity.

473.1


The anxiolytics, buspirone and gepirone, do not exert their actions until about two weeks. In this study we examined the effects of chronic gepirone administration on synaptic transmission in the CA1 region of rat hippocampus, and compared the acute effects of buspirone and serotonin. Mini-osmotic pumps were loaded with gepirone or with saline and implanted into the ventricular cavity to deliver 0.5 mg/kg/day. After 13 to 18 days, hippocampal slices were prepared for electrophysiological study using standard procedures. In slices obtained from rats treated with chronic gepirone therapy there was a reduction in frequency-dependent potentiation of both the presynaptic and postsynaptic components of the field potential. In control slices, bath applied buspirone (100 mM) led to a distinct reduction in the EPSP elicited by a single pulse of 1 ms at 5-10 Hz and an additional, non-rectified, presynaptic axonal excitation. Following chronic treatment with gepirone, the effects of acute buspirone application on both presynaptic and postsynaptic potentials were significantly reduced. Additionally, while serotonin (100 mM) had minimal effect on the postsynaptic potential and no effect on the presynaptic fiber volley in control slices, chronic gepirone treatment resulted in serotonin-evoked depression of the EPSP.

These results indicate that chronic treatment with gepirone reduces synaptic facilitation and leads to changes in the acute effects of buspirone and serotonin on synaptic transmission in the hippocampal slice. This implies that the delayed effects of chronic buspirone or gepirone treatment with respect to their anxiolytic action may be retained in the in vitro slice, thereby providing a system to study the long-term modulatory action of these agents on a cellular level.

473.2


Place preference conditioning and in-vivo microdialysis were used to examine the motivational effects of the serotonin (5-HT) agonist 8-OH-DPAT on locomotor activity in mice and rats. A slight but significant later-onset inhibition of locomotor activity was observed. These data demonstrate that 8-OH-DPAT is a secondary reinforcer and that this effect involves both 5-HT1A and 1-D1A receptors. Furthermore, they suggest an important role of the NAC and the 5-HT fibers projecting therein in the mediation of this effect.

Supported by the DFG and Bundesgesundheitsamt.

473.3


Depressed patients have a blunted prolactin (PRL) response to i.v. tyramine (TRP). A 10% reduction in plasma TRP following a low TRP diet and a TRP-free amino acid drink (AAD) produces a return of symptoms in 2/3 of depressed patients recently remitted on antidepressant medication. To evaluate the role of SHT in the blunted PRL release and the mechanism by which TRP depletion reverses antidepressant effects, the effects of TRP depletion on the PRL response to SHT agonists was compared to the effects of the SHT synthesis inhibitor paroxetine (PCPA). Adult male chair-trained rhesus monkeys received i.v. TRP at 200 mg/kg or the SHTIA agonist i.v. gepirone (GEP) at 25 mg/kg to stimulate PRL release. The AAD consisted of 15 essential amino acids with or without TRP added and was administered i. s. mg/kg through oral tube for 21 days. TRP depletion decreased plasma PRL by 30% following the AAD and PRO values were measured before and during the infusion with standard RIA methods. The AAD minus TRP produced an average of approximately 80-80% reduction in plasma TRP, along with an 80% reduction in PRL response to infused TRP, and a 40-60% reduction of PRO release to GEI. The AAD with TRP added also reduced the PRO response to TRP and GEP but to a lesser degree, 60 and 30% respectively, and PCPA at a total of 300 mg/kg over a 6 day period produced 29 to 40% reduction in the PRO response to TRP and a 25% to 200% increase in PRO response to GEP. The blunted PRO response to TRP following TRP depletion and PCPA and the augmented response to GEP following PCPA are consistent with prior data. The blunted PRO response to GEP following TRP depletion and the blunted response to both TRP and GEP following the TRP augmented AAD suggest that the metabolic changes induced by the amino acid lead to more complex alterations in SHT function than those observed following the more selective SHT synthesis inhibitor PCPA. Supported by MH16229 and MH125642.

Society for Neuroscience Abstracts, Volume 16, 1996
473.5 SECONdARY CHANG es IN THE EVOKEd RELEASE OF SEROTONIN FROM FRONTOCORTICAL NERVE TERMINALS INDUCED BY FLUOXETINE. A SEROTONIN RECEPTOR BLOCKER. A M. Cardier and R. J. Wurtman, Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Previous studies in our laboratory have suggested that the evoked release of serotonin (5-HT) is diminished in rats after chronic administration of fluoxetine, a 5-HT uptake blocker, or d-fenfluramine (d-fen), a drug which both releases 5-HT and blocks its reuptake. However, the treatment then chosen to evoke the 5-HT release was a drug, d-fen, for which it is not known to be by 5-HT uptake system in order to exert its effect. Thus, we decided to evoke 5-HT release by depolarizing 5-HT terminals with a high concentration of KCl, a process that uptakes. We gave rats either an oral dose of 2mg/kg/day). Fluoxetine (30mg/kg) or d-fen (75mg/kg) i.p. for 3 days and measured frontocortical 5-HT release in anesthetized rats using brain in vivo microdialysis 24 hours after the last dose. After extracellular frontocortical 5-HT levels stabilized, KCl 120mM was added to the perfusion fluid for 15 min. All samples collected for 15 min were assayed for 5-HT and 5-HIAA using LC-ED. Chronic fluoxetine decreased basal 5-HT and 5-HIAA release while chronic d-fen decreased 5-HIAA efflux only. Unlike the situation in d-fen pretreated rats, KCl-evoked 5-HT release was suppressed following fluoxetine pretreatment. This blockade of evoked 5-HT release, and of basal 5-HT and 5-HIAA release were not dependent on the dose of fluoxetine studied, producing after 7.5, 15, 30 or 60mg/kg. Although 5-HT and 5-HIAA release ultimately returned to normal, blockade did persist after a drug washout period of 7 days. These findings suggest that, unlike d-fen, fluoxetine can cause prolonged impairments in evoked 5-HT release.


Parenteral administration of D-fenfluramine to rats in massive doses (20-80 mg/kg/day for 4 days) has been reported to selectively remove 5-HT-immunoreactivity from axons in the neocortex and striatum up to 4 weeks post-treatment (Molliver and Molliver, Soc. Neurosci. Abst. 15:210,1989; Molliver and Molliver, Brain Res. 511:165,1990). Since these results have significant implications, we repeated this study using D-fenfluramine in orally administered (gavage) doses ranging from 2-48 mg/kg/day for 4 weeks post-treatment survival periods up to 2 weeks. 5HT-immunoreactive axons were examined in serial, sagittal, 30 um sections in identical regions of the neocortex of D-fenfluramined-treated rats and paired controls. At 24 hrs post-treatment, a progressive, dose-dependent reduction in 5HT-immunoreactive axons in the neocortex was observed. Doses of 12 mg/kg/day for 4 days produced the first evidence of abnormal 5HT-immunoreactive profiles. This dose (12 mg/kg/day for 4 days) and adjacent doses (8 & 16 mg/kg/day for 4 days) were used for the 2 week post-treatment study. Under these conditions neurochemical 5HT-immunoreactivity in the treated animals were comparable to controls. These results demonstrate that D-fenfluramine does not produce permanent loss of 5HT immunoreactive axons in the rat neocortex. Furthermore, the initial loss of 5HT-immunoreactivity does not appear to reflect neurotoxicity since the immunoreactivity reappears at 2 weeks post-treatment with a pattern similar to that in controls.

473.8 ERGOTAMINE IS A SPECIFIC INDUCER OF MOVEMENTS IN UNHATCHED ATLANTIC SALMON (Salmo salar) LARVAE. B. T. WALTER, J. V. HELVICK*, D. OPPEN-BERNSTEIN & C. BONG,* Dept. of Biochemistry, Univ. of Bergen, N-5009 Norway.

Exogenous neurochemicals advance or delay the time of hatching of salmon eggs (Oppen-Bernstein et al., 1980, AQUACULTURE 80, in press). After hatching light induces salmon larvae to initiate bursts of swimming. We tested a number of neurochemicals (40 mg/l) and found that only serotonin caused immediate and recurrent increases in movements of larvae inside eggs posed for normal hatching in daylight. Serotonin induced movements in all larvae after a lag in individual larvae most of a few seconds to 15 min at 4°C. Stimulation lasted about 30 min at 4°C, and about 60 min on ice. While normal movements of larvae involve slight rocking or at most a full rotation, serotonin induced 2-5 vigorous rotations. On average control larvae initiate such motions 1.1 times per hour. Serotonin induces a 26.7 fold increase in 2 more vigorous rotation motions, while tyramine, dopamine, noradrenaline, adrenalin, histamine and melatonin had no effect. Weak stimulatory compounds were found: tryptamine 3.2x increase, 3-acetamidoserotonin, 3-methoxytyramine, and 5-hydroxytryptophane both 2.2x increase over control. In these salmon eggs close to term only tyramine appeared to accelerate treated eggs all hatched 24 hours after our test, except the adrenergic-treated eggs. Stimulation of hatching movements in salmon larvae is driven from stimulation. Supported by NFFR & Nordic Industrial Fund.

473.6 STUDIES WITH NEUROTOXIC AND NON-NEUROTOXIC ANALOGUES OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA). M. E. Johnson and D. E. Nichol. Deps. of Pharmacology and Toxicology, and Medicinal Chemistry and Pharmacodynamics, School of Pharmacy and Pharmaceutical Sciences, Purdue Univ., Lafayette, IN 47907.

It has previously been reported that rigidification of the α-side chain of p-
chlorophenyl (PCA) to give chloro-2-aminoetanol (6-CAT) ammutes the serotonergic neurotransitivity of the parent compound (Fuller, R. W., et al, Arch. Int. Pharmacodyn. Ther., 212:141, 1975). This led us to the synthesis of rigid analogs of MDMA that retained the behavioral activity but lacked the apparent long-term neurotransitivity of the parent compound (Nichols, D. E., et al, J. Med. Chem., 35:703, 1990). One of these rigid analogs is 5,6-methylenedioxy-2-adaminomethan (MDA). The levels of monoamines at short time periods after high doses of MDMA and MDA were examined. In the frontal cortex and caudate of rats, there are significant differences in the effect on DA and its metabolites with the neurotoxic and non-
neurotoxic amphetamine analogues. This report also describes several additional analogues of MDMA as well as short-
term and long-term effects of these analogues. For example, the importance of ring substitution was investigated by examining 3-methoxy-4-methylamphetamine (MMA) and 5-methoxy-6-methyl-2-adiminomethan (MDMA). The serotonergic neurotransitivity of these compounds was determined by examining [3H]paroxetine binding and monoamine levels one week following acute doses of 10 or 20 mg/kg i.c. or two weeks following subacutone studes (daily for 20 days) of 20 mg/kg. None of the treatments led to changes in serotonergic markers with either MMA or MDMA. These neurotoxic and non-neurotoxic substituted amphetamines were examined for their relative potencies in a number of bioassays. The relative ability of the amphetamines to release and to inhibit the uptake of 3H-5HT, 3H-DA and 3H-NE was examined. The results will be discussed in terms of the relative importance of these short-term actions on the long-term effects of certain substituted amphetamines. This work was support by USPHS grant DA-04358 from NIDA.
46.1 ALTERATION OF NEURONAL REGULATION OF ASTROCYTOMA PROLIFERATION BY INSERTIONAL MUTAGENESIS B.A., T. BIBBE, R.H. LIEE AND M.L. SHIELANSKI, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, NEW YORK CITY, N.Y. 10032.

It has been previously shown that neuron-astrocyte cell contact results in the arrest of astrocyte DNA synthesis and that this inhibition is mediated (Hatten, M.S. and Hajos, P.G. J. Cell Biol. 104: 1533-1537, 1987). In addition, coreillary granule cell membranes will inhibit the growth of astrocytoma cell lines derived from rodent and man (Hatten, M.S. and Shielanski, M.L. J. Neurosci. 8: 1447, 1988). In the current experiments, we report the retroviral shuttle vector pZIPneoSV (X) to create a selectable alternative (insertional mutation) in a mouse astrocytoma cell line (G-264), which normally grows in the absence of granule cell membranes. Successfully infected G-264 mouse astrocytoma cells were isolated by G418 selection. These were then again selected for retention of inhibition by reconstituting neurons and neuronal neurite outgrowth. Of several hundred G418 resistant clones that were isolated one, RM-31, shows significant resistance to neuronal inhibition of DNA synthesis. Indirect immunofluorescent staining for GFAP is strongly positive in both wild-type G-264 and clone RM-31, with wild-type astrocytic morphology preserved. Quantitation of phenotypic effects by FACS analysis and by cell count show that RM-31 has an increased growth rate (1.5 X wild-type) and that neuronal inhibition is reduced to 15% that of the wild-type G-264. 3M-Smethionine labeling and extraction decreases a decrease in the protein synthesis of 210, 110 and 90 kD. Southern blot analysis confirms that we have isolated an astrocytoma mutant in which regions of DNA, normally encoding for proteins which mediate the neuronal regulation of astrocyte DNA synthesis have been altered or deleted. This lesion could either be at the receptor for the neuronal or the astrocytic membrane, or at any point along the complex cascade of events regulating cellular proliferation. We are currently in the process of isolating the segment of the viral genome by inverse Polymerase Chain Reaction (Supported by NS 21457).

47.4.2 EGF- AND TGFα-RESPONSIVE STRIATAL EMBRYONIC PROGENITOR CELLS PRODUCE BOTH NEURONS AND ASTROCYTES. B.A. Remold, W. Tetzlaff and S. Weiss, Neuroscience Research Group, Univ. of Calgary, Calgary, Canada.

The effects of EGF on proliferation of clonally isolated embryonic CNS progenitor cells were tested by plating E14 mouse striatal cells (1250 cells/cm²) on poly-l-ornithine coated coverslips in defined, serum-free media. Division of progenitors (150 cells/cm²) was first observed at 5-7 days in vitro (DIV) and by 14 DIV, these dividing precursors formed proliferating clusters. Untreated cultures showed no such proliferation. Indirect immunocytochemistry with each of specific antibodies (NSE, antifibrillar acidic protein (GFAP) was utilized to identify neurons and astrocytes, respectively. At 14-21 DIV, NSE- and GFAP-immunoreactive (IR) cells were found in the proliferating core with some IR cells migrating from the center. After 21-35 DIV, a large number of cells were GFAP-IR and stellate in appearance; many of the GFAP- and NSE-IR cells had migrated from the proliferating core. While the majority of cells were GFAP- and NSE-IR, a large number of rounded cells with multipolar morphology were negative for both markers. EGF and TGFA effects were similar, yet in TGFα-treated cultures the NSE-IR cells displayed elaborate processes and non-stellate GFAP-IR cells were present. These findings also show that the same CNS precursors in vitro to produce both neurons and astrocytes. Supported by the Medical Research Council of Canada.

47.4.3 POSTEMBRYONIC NEUROGENESIS IN THE BRAIN OF MANICD4 SIX7α/α. K.A. Sorenson, N.T. Devos, and J.J. Hildebrand, ARU Division of Neurobiology, University of Arizona, Tucson, Az 85721.

The nervous system of insects that undergo complete metamorphosis is disarmingly simple in the transition from egg to adult. One aspect of this reorganization is the postembryonic addition of new neurons to the embryonically derived larval nervous system by division of neural stem cells termed neuroblasts. (Wilhelm Roux’ Arch. 164:247 (1970); J. Comp. Neurol. 225:548 (1987); Dev. Biol. 125:145 (1988)). We are studying the origins of the neurons of the primary olfactory center, the antennal lobe (AL), in the brain of the adult Manduca sexta. We have found that the contribution of postembryonic neurogenesis to the formation of the AL, we used the thymidine analog 5-bromo-2-deoxyuridine (BrdU), which selectively labels the genome of mitotic cells, to reveal proliferative neuroblasts and the time course of their appearance. Incorporation of BrdU was achieved by feeding larval normal diet in which BrdU had been added at 0.05 to 0.1 mg/ml of the incorporation nucleotide was then visualized at successive stages of development by means of whole-mount immunocytochemistry employing anti-BrdU antibody and PAP. Scattered neuroblasts were first identified at mid-first instar and in the level of staining over the course of the remaining stadia. Labeling is seen first in the brain and subsequently in the subesophageal ganglion and segmental ganglia, developing in an apparently anterior-to-posterior temporal gradient. Staining appears in all ganglia by the beginning of the second larval instar. Whole-mount preparations reveal 3-7 pale putative neuroblasts that lie close to the larval antennal center and thus may contribute to its metamorphosis into the adult AL. We will use sectioned preparations and cell lineage tracing techniques to ascertain cellular relationships and fates.


The present experiment assessed the developmental window during which neurogenesis of the amygdaloid complex takes place in the fetal primate brain. Nine pregnant rhesus monkeys received an injection of tritiated thymidine between embryonic [E] days 27 and E56 of their 165 day gestation. Offspring were sacrificed in the postnatal period and 8 μm coronal brain sections. Histology and immunocytochemistry were used to analyze the origin and differentiation of the amygdaloid complex and its associated structures. Neurons were labeled with GFAP and neuronal nuclei with antibodies to tubeulin and identified by immunohistochemistry. Only neurons were present within any amygdaloid nucleus on E27. A few labeled neurons were observed in the case exposed to tritiated thymidine on E30 making the amygdala, concomitant with the magnocellular basal forebrain nuclei, the earliest developing structure in the telencephalon. At E33, significant labeling was principally located within the central and medial amygdaloid nuclei. A few heavily labeled neurons were present within the lateral nuclear groups as well. Significant neurogenesis was observed at E34 for all amygdaloid nuclei. Antibody to GFAP and tubeulin identified the amygdala, which begins during the second trimester of pregnancy, suggests that the actual pattern of cellular development occurs across a medial to lateral gradient. [AHAH, NS 26555; HK, NS 14481 PR].

47.4.5 CELL CYCLE KINETICS OF THE E14 MURINE CEREBRAL VENTRAL ZONE: ESTIMATES BASED UPON S-PHASE LABELING WITH BUdR. T. Tabakhan, M. Jacobson, R. S. Nowakowski and V. S. Caviness, Jr., Dept. of Neurological Surgery, Chicago, Yale, Conn., and M.D., and Dept. of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Proliferating cells in S-phase in the cerebral ventral zone (V2) of E14 mouse embryos (EO = day of conception) were labeled cumulatively with BUdR over 2 hr. Embryos were harvested at 0.5, 2, 0.5, 3.5, 6, 8, 9, 12.5 hr following the initiation of labeling. After immunization and overnight fixation in 70% ethanol, the brains were embedded in paraffin, sectioned at 4 μm, stained with hematoxylin and eosin, and were analyzed with a fluorescent microscope (FL). Both the mitotic index (MI) and the percent of BUdR labelled (L) for the V2 increased approximately linearly from 0.5 hr a value of 0.25 (extrapolated to 0.25 at 0.5 hr) to 1.0 after an estimated interval of 10.0 hr. The growth fraction is thus 100%, which means that all cells of the V2 are proliferating. The linear increase in L implies that there is only a single proliferative population. A maximum value for the length of the overall cell cycle (Tc) is computed to be 13.8 and S-phase (Ts) 2.7 hr. Since labeled mitoses were observed 2.0 hr after the initiation of BUdR labeling for the G2+U portion of the cell cycle, the duration of the S phase was estimated to be 2.0 hr. With the exception of Ts, these values fall within ranges estimated by cumulative δH-thymidine labeling in rat embryos of comparable age (16E) by Waeschler and Jaesch (Brain Res. 46: 235-250,1972): Ts = 2.8 - 6.5 hr, Tc = 8.6 hr, To-M = 2.5 hr. Among the considerations that might explain the Ts discrepancy with the S-phase is that the thymidine labeling method or the possibility that the δH-thymidine had been available to label S-phase longer than a few minutes. This may be due to differences between the two species.
474.7

The hypothalamus has been studied in the pig since the timing of its brain growth spurt in relation to birth and gonadal steroid levels would be comparable to that of human beings. In the present paper the results of a morphometric and immunocytochemical study of a vasopressin and oxytocin containing nucleus in the pig hypothalamus are presented. This nucleus has not been described before. Neuron numbers and volumes were measured at four different ages i.e., 1 day, 7 weeks, 16 weeks and 30 weeks postnatally with a computer assisted method. There were no sex differences found at any of the ages studied. At birth an average of 1200 neurons is found, in the first 7 weeks postnatally the number decreased to 770. A striking finding was that between 16 and 30 weeks (i.e. during puberty) an increase from 700 to 1800 (260%) neurons is seen. Immunocytochemistry (antibodies were a gift from Dr. A. Hou-Yu, Columbia University, New York) revealed vasopressin and oxytocin immunoreactive neurons. This increase in the number of vasopressin and oxytocin containing neurons in the pig hypothalamus is much later in development than has been reported so far.

474.9
USE OF PCR TO IDENTIFY PRE- AND PERINATAL LETHAL MUTANTS: APPLICATION TO HOMOZYGOUS LURCHER MICE. J. M. Soba and K. Hernp, E.K. Shriver Center, Waltham, MA 02254.

Lurcher is an autosomal dominant mutation located on chromosome 6 of the mouse. Heterozygote (+/Lc) animals become ataxic during the first postnatal month in association with the loss of 100% of their cerebellar Purkinje cells as well as other presynaptic neurons. The homozygote condition (Lc/Lc) has never been described and is presumed to be a pre- or perinatal lethal. To identify Lc/Lc embryos and, later, Lc/Lc vs +/+ chimeras, a molecular assay has been developed. C57BL/6 and AKR/J mice exhibit a HinFl restriction fragment length polymorphism in the Ig-k locus about 2 centiMorgans from the Lc locus. AKR/J males were crossed with C57BL/6 +/+ females and the F1 +/+ mice were intercrossed. In the resulting F2 generation, PCR amplification of a 820 bp fragment around the HinFl site, followed by HinFl digestion, lead to a positive identification of any fetuses or newborn pup (with about 96% certainty). Homozygous (Lc/Lc) animals have only the C57BL/6 form of the polymorphism, while heterozygotes (+/+Lc) animals have both.

At E14, Lc/Lc embryos identified by this method exhibit cerebella that are clearly smaller in cross-section in both the sagittal and coronal planes compared to either +/+ or wild type embryos. Cerebellar volume is decreased by about 40%. Development of folia is also severely retarded. In addition, a greater incidence of pycnotic profiles is seen among cells of the cortical plate at the HinFl embryos.

Supported by NIH grants NS18381 and 230991 (KH), NS8896 (JS), and by the March of Dimes 1-1175 (KH).

474.10
ROLE OF ELECTRICAL ACTIVITY IN THE DEVELOPMENT AND SURVIVAL OF CULTURED HYPOTHALAMIC NEURONS. D.S. Ling, R.E. Pekris, and H.M. Geller, Departments of Pharmacology and Biomedical Engineering, UMDNJ-Robert Wood Johnson Medical School and Rutgers University, Piscataway, NJ 08854.

Hypothalamic neurons exhibit a gradual, consistent increase in spontaneous action potential discharge in long-term culture. To examine the role of electrical activity on neuronal bioelectrical development and survival, we have examined the effects of tetrodotoxin (TTX) and high KCl on cultured hypothalamic cells.

Disassociated neurons from E17 rat hypothalami were plated onto a layer of cortical astrocytes in either microwell trays or on glass coverslips. Starting at 1 day in vitro, cultures were treated with TTX and/or high KCl. Neurotransmitter survival was assessed by counting cells on an inverted phase microscope. The development of spontaneous action potentials was examined using an extracellular loose patch recording technique. Cell counting and electrophysiological experiments revealed that TTX caused a marked reduction in both cell survival and the percentage of spontaneously active cells as compared to controls. Concurrent treatment with high KCl inhibits the deleterious effects of TTX on cell survival and the development of spontaneous activity.

When taken together, the results of these experiments suggest that the suppression of electrical activity selectively increases the death of spontaneously active hypothalamic neurons and that the long-term survival of spontaneously active cells may depend on continual membrane depolarization. (Supported by NIH NS 25168)

474.11

Holometabolous insects must undergo complete reorganization to change from larval form to adult form. Hormone release, temporally and quantitatively, plays an integral role in controlling this complex process. Larval muscles and neurons respond to varying levels of specific hormones; degeneration of these tissues occurs in response via programmed cell death. I have observed a large (6x13 um), unique cell type appearing in the thoracic region during early metamorphosis in Drosophila which plays a role in muscle degeneration. These cells become prevalent in the early prepupa (5h), first appearing at ventral sites and then migrating dorsally along body wall muscles. Each cell attaches to a muscle prior to any sign of degeneration in the muscle itself. Subsequently, the cell extends processes into the muscle and degeneration of the muscle region occurs. This event occurs progressively from the prothoracic region to the metathoracic region. The migration of these cells to the muscles happens through an active identification process. Although most thoracic body wall muscles are attacked by the "killer-cells", a few adjacent muscles lack the cells. These persistent muscles serve as substrates for adult myocytes which will form all of the adult indirect flight muscles.

Such "killer-cells" appear to be another important factor in programmed cell death of muscles during metamorphosis. Their ability to avoid selectively the proper recognition process by these cells. The basis of this selection and the hormonal control of these cells' activity are being studied.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
475.1
MARKERS OF NEURAL MATURATION IN NEUROBLASTOMA CELLS TREATED WITH NEOCARZINOSTATIN. N. F. Schor, M. R. Gilbert, J. Leventhal, and C. F. Lagunam. Children's Hospital of Pittsburgh, Pittsburgh, PA 15232. The Johns Hopkins Hospital, Baltimore, MD 21205, and University of Pittsburgh, Pittsburgh, PA 15261.

Treatment of murine neuroblastoma cells with the antineoplastic natural product neocarzinostatin results in morphological changes suggestive of differentiation. One extended processes, stop dividing, and, unlike their "immortal" untreated counterparts, die after 14 days in culture. We have looked at these cells for the presence of several cell markers of neural differentiation. Both bi-laminar cell cultures and hippocampal cultures were treated with neocarzinostatin. In cells treated with neocarzinostatin for 1 hour on day 0 express nerve cell adhesion molecule (NCAM) by day 14 as brightly stained cell surface staining with fluorescent polyclonal antibodies to NCAM; untreated cells do not stain for NCAM. Staining with antibodies directed against L1 or HNK-1 does not change in these antigens upon treatment of neuroblastoma cells with neocarzinostatin. Both L1 and HNK-1 are cell adhesion molecules which share a common carbohydrate epitope with NCAM. Electron microscopy, histochemical staining, and Northern blotting indicate that neocarzinostatin is not present in treated or untreated cells. This shows that the processes extended by neuroblastoma cells are not normal axons, and that the morphologic differentiation induced by neocarzinostatin is not indicative of "normalization" of neuroblastoma cells.

475.2

Temperature-sensitive alleles of the SV-40 large T-antigen have been used to immortalize hippocampal precursor cells in culture. The results of a recent study indicate that the resulting cell lines have altered function of the EGF receptor (EGF-R) and may be potentiated for neuron-glial differentiation. Immunostaining for hippocampal precursor cells (NFP, GFAP, and 3) have been visualized by immunostaining in several NFP- lines. The former has been reported to be present in the dendrites and the latter in the axons of mature neurons. In developing neurons both are distributed throughout the cell. In our cell lines these markers tend to be asymmetrically distributed in the perinuclear region except in the most differentiated (by morphology) cells. Since the proportion of cells undergoing morphogenetic differentiation and the combinations of the labeled markers vary from line to line it is likely that we have immortalized several different types of hippocampal precursor cells. (Supported by NS-25787 and the Alzheimer's Disease and Related Disorders A.)

475.3

Epidermal growth factor (EGF) is a potent mitogen for fibroblast and epithelial cells. In order to characterize the EGF receptor in a nonproliferating cell system, we utilized hippocampal cultures capable of terminal differentiation. We had previously shown expression of EGF receptor in primary hippocampal cultures by immunostaining. In order to cell line differentiation, serum failed to stimulate DNA synthesis, although the retraction of cell processes was observed. We cultured several cell lines for EGF receptor activity by binding EGF before and after differentiation. One line, WH1-9, exhibited a 2-fold increase in binding activity while another line, H19-7, increased its binding activity 8-fold after differentiation. Experiments in which these two cell lines were transfected with the EGF receptor promoter suggest that the changes in EGF receptor binding result from changes in receptor transcription. Thus, these two hippocamal cell lines provide powerful tools for studying both positive and negative regulation of growth factor receptor transcription during hippocampal cell development, as well as allowing us to investigate nonmitogenic cell signaling pathways by the EGF receptor tyrosine kinase. (Supported by 5 T32 GM 07151-14 and International Life Sciences Institute.)

475.4

A rat pheochromocytoma cell line, PC12, was co-cultured with dissociated Schwann cells (SC) from sciatric nerves of 2 day old rats. SC were usually seeded first. They spindle shaped and formed clusters of closely spaced cell bodies with processes radiating outward. Naive PC12 cells, when added, randomly settled initially, then rapidly migrated toward the clusters of SC. By 3-4 days, 60-80% of the PC12 cells were aggregated on top of the SC. Differentiated PC12 cells, e.g., nerve growth factor (NGF)-primed or K-ras virus infected, exhibited this affinity towards SC even earlier by 24-36 hrs in co-culture. There was no aggregation of PC12 cells over contaminating fibroblasts in the SC preparations, or over other types of control cells, such as astrocytes, brain and aortic endothelial cells. The close PC12-SC association consisted of PC12 cells stacked, sometimes 5-8 cells deep, on top of SC. Ultrastructurally, in so SC cultures, the cells usually contained many intermediate filaments (IF) than did SC in vivo. Sometimes, the IF were packed in crystalline form in the cell bodies. Microtubules were prominent in the cell processes. In co-culture with PC12 cells, some SC underwent differentiation: an increase of rough endoplasmic reticulum, a decrease of intermediate filaments and enhacement of PC12 neurites by flat SC processes. The wrapping of PC12 neurites by sciatic nerve SC in is contrast to the report of non-endothelial from SC dorsal root ganglia. Conversely, both SC and PC12 derived extracellular matrix supported neurite extension from PC12 cells. These neurites contained abundant microtubules, dense core granules (100 nm) and small, clear vesicles (50 nm). In these co-cultures, the production of choline acetyltransferase, assessed biochemically, increased by 50-100% while that of acetylcholinesterase was largely unchanged.

475.5
COMPARISON OF TOTAL CELL PROTEIN PRODUCTION IN ORGANOTYPIC CULTURES FROM MOUSE SPINAL CORD WITH THAT IN INTACT MICE. L. T. Stewart, J. A. Bird*, N. A. Mills*, and M. H. Diogen. Dept. of Biology, Texas Woman's Univ., Denton TX 76204.

The goal of our research is to establish an in vitro mammalian spinal preparation suitable for investigating the mechanisms underlying behaviors. A cell culture system currently under investigation involves organotypic cultures from 300 µm transverse sections of neonatal mouse lumbar spinal tissue. This study has recently shown that relative densities and quantified by morphometric indices between the cultured tissue and that of intact mice at similar postnatal ages. This study compares morphology and total cell protein production in both preparations.

Lumbar spinal tissue obtained from neonatal mice was sliced and established in roller drum culture; fresh, noncultured tissue was similarly obtained and sliced. At similar postnatal intervals, intervals, both noncultured, and cultured samples were homogenized in medium containing 35S-methionine and 3H-thymidine and examined for 1 precipitable counts via scintillation counting, and 2) protein patterns from electrophoretograms. The current study compared morphological and total cell protein production in both preparations.

Lumbar spinal tissue obtained from neonatal mice was sliced and established in roller drum culture; fresh, noncultured tissue was similarly obtained and sliced. At similar postnatal intervals, samples from each group were incubated in medium containing 35S-methionine and 3H-thymidine and examined for 1 precipitable counts via scintillation counting, and 2) protein patterns from electrophoretograms. The current study compared morphological and total cell protein production in both preparations.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
475.7 DIFFERENTIATION MODULATION OF PROTEIN KINASE C ISOYME DIFFERENTIATION IN PC12H CELLS STIMULATED BY DBUTYRYL C-AMP. S. Shimada, Y.U. Kusunoki, H. Tamura, T. Taniguchi, H. Ninomiya, and T. Saito*, Dept. of Neurology, Kyute Univ. Sch. of Med., Dept. of Neurobiology, Kyute Pharmaceutical Univ., Kyute 606, Japan, Dept. of Neurosciences, Univ. of California, San Diego, La Jolla, CA 92037, USA.

PKC is a family of closely related enzymes, and the role of each PKC isoyme remains unknown. Rat pheochromocytoma PC12 cells can be induced to differentiate into cells with typical neuronal morphology by a number of substrates, however, the molecular mechanisms are not clear. To clarify the different functional role of each isoyme, we examined differentiated morphology of four types of isoymes in the differentiation of PC12H cells by using immunoblotting analyses. We have employed four anti-PKC antisera raised against C-terminal variable region of PKC(a), (b), (bII), and (9). Two types of PKC isoymes, a and bII were predominantly detected in PC12H cells. When exposed to imd dibutyryl cAMP, PC12H cells clearly began to exhibit the neurite outgrowth at around 30 hrs. Neurites looked narrow and unstable. After 24 hours, these neurites became much stable and wide ribbon-like shapes. When immunoblotting was carried out for the homogenates of dibutyryl cAMP-stimulated PC12H cells, we found the prompt and temporary accumulation of PKC(a), whereas the accumulation of PKC(bII) was prolonged for nearly 24 hours following the addition of dibutyryl cAMP. These results suggest that PKC(a) and PKC(bII) may be involved in different cellular processes and regulate the specialized physiologic responses in the differentiation of PC12H cells and that concerted action between protein kinase C and cAMP(secondary) dependent protein kinase systems may work on the differentiation process of PC12H cells.

475.8 SURVIVAL AND DIFFERENTIATION OF PURIFIED PURKINJE CELLS FROM MOUSE CEREBELLUM C.A. Baptista, M.E. Hatton, and C.A. Mason, Dept. Pathology, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

In our studies of axon-target interactions in vitro (Baird et al., this volume), we have shown that purified cerebellar granule neurons specifically signal their appropriate targets, the optic mossy fibers, to stop growing. To examine axon growth cone behavior and interactions with Purkinje cells, we have developed affinity methods to purify Purkinje cells, and have developed affinity methods to purify Purkinje cells. We have used affinity methods to purify Purkinje cells. We have developed affinity methods to purify Purkinje cells. We have used affinity methods to purify Purkinje cells. Although the yield of Purkinje cells was very high with this method, neurite outgrowth and cell polarity were impaired, and survival was limited to 1-2 days. In contrast, in the presence of glial cells and other neurons, Purkinje neurites survived for up to two weeks, and developed long axons and elaborated characteristic dendrites. Studies are in progress to identify the factors (substrates, growth factors, and surface molecules) critical to the survival and differentiation of purified Purkinje cells. This culture system will in turn allow us to test the specificity of axon-target interactions, and the effect of olivary (climbing fiber) afferents on Purkinje cell morphogenesis. Supported by NS 16981.

476.1 PROTO-ONCOGENE PROTEIN EXPRESSION DURING MOUSE BRAIN DEVELOPMENT. J.B. Hatchim, Department of Anatomy, University of Mississippi Medical Center, Jackson, MS 39216-4505.

Proto-oncogenes and their protein products are believed to play a key role in mammalian development. Proteins transcribed from proto-oncogenes are known to regulate the expression of numerous genes, and by this mechanism they may determine a cell's fate. The pattern of expression of the growth factor PDGF (which can exist in AA, BB or BB dimers) as provided by the proto-oncogene products Myc and Sim (homologous to platelet-derived growth factor B chain, PDGF-B) were studied during prenatal mouse CNS development (embryonic days 10 through 16, E10-E16). All three proteins have been implicated in control of CNS cell lineage. Expression of PDGF-like immunoreactivity has been found by the earliest time point studied (E10 spinal cord, E12 retina). Long, thin, spike-like processes extend from these labeled cells in both regions. However, in pros, meso, and rhombencephalon, PDGF-like cells are still round and clustered in the ventricular zone. Later, PDGF-like cells in all three encephalic regions take on a more differentiated appearance. No appreciable PDGF immunoreactivity is seen in the brain of juvenile or adult animals. Myc-like immunoreactivity in the developing CNS is not seen until about E15. In the E15 retina, Myc-like nuclei cluster in the developing ganglion cell layer, near the vitreal surface. No Myc-like nuclei are seen in the presumptive outer layer of the retina. Myc-like nuclei are scattered throughout the spinal cord at E16 in an apparent random distribution.

The pattern of sis-like immunoreactivity is similar to that of Myc in both time course and distribution. The distribution of Sis-like cells is strikingly different from cells labeled with PDGF. The specificity of the PDGF antisum is still being explored, but may reveal interesting disparities between the localization of Sis/PDGF and the putative distribution of AA or BB dimers. Supported by NSRGR R03806 (to U.M.) and EY00828 (to J.B.H.).

476.2 A ROLE FOR IC IN REGULATING ACTION POTENTIAL DURATION IN HIPPOCAMPAL NEONATAL NEURONS OF RABBITS. J.V. Sanchez-Andres and D.L. Alkon, Laboratory of Molecular & Cellular Neurobiology, NIH, Bethesda, MD 20892.

Duration of the action potentials (AP) in CA1 cells of hippocampus in developing cats is prolonged when compared with adults. This effect has been attributed to the slower rate of repolarization (Papura et al. 1968). Schwartzkroin (1982) confirmed this observation in newborn rabbits, and observed that the APs showed a duration similar to that of adult cells by about 10 to 14 postnatal days. Handel and Rettig (1989) proposed that the last two thirds of the AP repolarization phase are generated by IC in CA1 neurons. From the above, we propose that IC is incompletely developed in neonatal neurons. We recorded both IC and Iapp (Ca2+ dep K+ currents) in CA1 neurons from newborn (1st-8th days after birth) and adult rabbits using a single fiber voltage-clamp technique. The IC values for the neonate were clearly reduced (M+SEX10 = 4.06+0.29, n=16) compared with the adults (5.832.50, n=18), (p<0.01, unpaired, two tailed t test). The rising phase of Iapp, usually undetectable in neonates, was present in the adults, although there were similar differences in IAPD amplitudes. These data a suggest that a small IC is responsible for the longer duration of the AP in neonates, and raise the possibility that neonatal CA1 K+ currents are not yet affected by learning-induced reductions as previously demonstrated for adults (Coulter et al., 1988; Sanchez-Andres and Alkon, 1989).

The subplate region of the developing cerebral cortex is believed to play a crucial role in the development of the deep cortical plate. We report here a broad characterization of the subplate region of the rat, both in vivo and in vitro. Immunocytochemistry, histochemical and (3H)-thymidine autoradiography techniques were used to analyze the development of the subplate region in frozen sections and organotypic slice cultures (Annis et al., '96) of fetal & newborn rat pups of varying age (E19-P14). (3H)-thymidine autoradiography, in sections of parieto-occipital cortex, indicates that subplate neurons in the rat are born between E13-14 and an identifiable subplate zone can be detected to adulthood. Immunocytochemistry with antibodies against GABA, NPY, Somatostatin, and VIP indicate that populations of subplate neurons both in vivo & in vitro stain positively with these antisera. Histochemical data indicate that a population of subplate neurons also stain strongly for acetylcholinesterase, in vivo & in vitro. These cells are horizontally or vertically oriented bipolar & multipolar neurons. A subpopulation of these AChE positive neurons demonstrate pseudopodial morphologies. Comparison of these data with those obtained by other investigators in cat and primate indicate strong similarities between the subplate neurons of the rat and those of other mammals.

Supported by NSF grant 87-08515 and NIH grant NS 25674.


Previous studies using a molecular marker of limbic phenotype, in combination with brain tissue transplants, has shown that there is an early critical period for irreversible commitment of developing cortical neurons to exhibit limbic features. The present study was undertaken to determine whether there is also an early specification of projection phenotypes in the limbic or neocortical transplants. Limbic (perihinal) and nonlimbic (sensorimotor) fetal tissue was transplanted into P1 rat host perihinal or sensorimotor cortex. Animals were sacrificed 2 weeks post-transplant and DI crystals precipitated onto pieces of finely-pulled glass micropipettes inserted into the transplant area. The brains were incubated at 37°C for 6-7 weeks. Affluent and effluent projections of hypothalamic and hypothalamic transplants were analyzed. Di labeling of perihinal cortex transplanted into sensorimotor cortex retrogradely labeled limbic-associated thalamic nuclei (e.g. lateral dorsal, parafascicular), as well as normal limbic thalamic nuclei (e.g. VM, VPL). In addition, Di-labeled efferents from the transplants could be traced to similar nuclei in the thalamus. Perihinal transplants into perihinal regions show exclusively limbic labeling. Sensorimotor transplants to sensorimotor cortex did not show any limbic projections. Experiments are in progress to examine heterotypically transplanted sensorimotor into limbic cortex. The data suggest that while molecular specification is under relatively strict regulation, phenotype as defined by projection patterns are subject to more complex environmental influences. Supported by NIH grant MH 45507.

416.7 Brainprints: Inter- and Intra-Observable Reliability M.J. Jouandet, W.C. Esslen, G.L. Weaver, M.S. Gazdzinski. Program in Cognitive Neuroscience, Dartmouth-Hitchcock Medical Center, Hanover, NH 03756.

We recently developed a new in vivo method of unlabeled, mapping, and measuring the human cerebral cortex using a line-drawing technique computer reconstruction of magnetic resonance images (Jouandet et al. 1989; J Cogn Neurosci 1:18-17). In order to consider the error inherent in our reconstruction procedures, intra- and inter-observer reliability were assessed by having four observers independently map one hemisphere and a fifth observer map one hemisphere three times.

Among the four observers, three had surface hemisphere area (SA) ranged from 930-1067cm², frontal lobe SA 229-249cm², parietal lobe SA 219-246cm², temporal lobe SA 193-222cm², occipital lobe SA 152-185cm², and limbic-parietal cortical SA 134-160cm². An inter-observer reliability coefficient of variation (CV) of each region of interest (ROI) SA measurement was calculated by dividing the population standard deviation by the population mean.

In addition, pair-wise correlations across ROI SAs for the four observers were calculated. For total hemisphere, frontal, parietal, and temporal SA, CV was 3.4%, 3.5%, 6.4%, 2.7%, and 1.5%, respectively. Among the 27 ROIS, SA measurements showed the least variation of any of the group. parietal and premotor triangles (3.0%) and the greatest in the superior lateral occipital cortex (8.4%). ROJ median CV was 6.4%. Pair-wise correlations ranged from 95-99.6%. CVs were also calculated to estimate intra-observer reliability. For total hemisphere, frontal, parietal, and temporal, and occipital SA, CV was 2.7%, 2.1%, 3.7%, 4.4%, and 8.3%, respectively.

These results compare favorably with accuracy estimates of regional cortical SA measurements obtained using the contour method of flat-mapping (a microtome animal cortex (Van Essen and Maunsell 1980) and indicate that our brainprinting technique yields reasonable, quantifiable data.

Supported by NINDS ROI NS 00014-07 (IT & MSG) and AFOSR 89-0437 (MSG).
647.8 RADIAL GLIAL IMMUNOREACTIVE FIBERS IN THE REGION OF SPONTANEOUS MICRODYSENEGESIS IN NEWBORN NEW ZEALAND BLACK MICE. G.E. Weissman, D.M. Praneth, and A.M. Guimarães. Department of Neurology, Harvard Medical School, and Beth Israel Hospital, Boston, MA 02118.

The New Zealand Black mouse strain (NZB) spontaneously develops severe autoimmune disease which produces prenatally deceased at about 16 months of age. In adulthood, these animals have cataracts, severe diabetes, and lesions in layer I of the thalamus and underlying portion of the cortical layers (Sherman et al., Acta Neuphotol. 74:339-342, 1985). Studies have shown a migration of cells into the area during postnatal period, that extends from the lateral ventricle to the subcortical layer I. These cells are similar to the cells that migrate into the normal thalamus, but they are less numerous in the normal thalamus.

Newborn NZB pups were transcardially perfused with 4% paraformaldehyde, the brains removed and post-fixed in the same fixative for 24 hours. The brains were then sectioned sagittally and transversely and stained with myelin-staining and immunohistochemical methods. The results showed that there was increased density of radial glial fibers in the area of the thalamus, but they were not as densely packed as in the normal thalamus. The results indicate that the migration of these cells into the area may be a result of the pathologic changes in the thalamus, rather than a compensatory response to the disease.
DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: POSITION AND FORM

476.15

EFFECTS OF PRENATAL COCAINE EXPOSURE ON BRAIN METABOLISM IN THE 21 DAY OLD RAT. D.L. Dow, Edwards, L.A. Fried, L.M. Donohue*, and H.E. Hughes, Laboratory of cerebellar Metabolism, Department of Neurosurgery, SUNY Health Science Center, Brooklyn, N.Y. 11203.

Human infants prenatally exposed to cocaine exhibit growth retardation and neuromotor and cognitive impairments. Recent animal studies suggest that cocaine directly affects brain development. We have observed long-term changes in functional brain regions in several brain regions in the offspring at birth (gestational days 8-22) and postnatal (days 1-10 or 11-20) cocaine exposure. The present study investigated rates of glucose utilization in various brain structures of weanling rats prenatally exposed to cocaine in comparison with control offspring.

Sprague-Dawley rats were gasticly intubated with 30 or 60 mg/kg/day cocaine HCl or vehicle only during gestational days 8-22. Vehicle treated rats were pair-fed to rats receiving the higher dose of cocaine. A non-treated control group was also maintained. At parturition, litters from all four groups were segregated. Rates of cerebrocortical glucose utilization were determined in 21 day old male and female offspring using the deoxyglucose method of Sokoloff et al. U. Neurochem, 28:97, 1977. The effects of prenatal cocaine exposure on patterns of brain metabolic activity as well as physiologic variables such as blood pressure, hematocrit and body temperature in 21 day old rat pups will be presented.

Supported by ADAMHA Grant # DA04118.

476.16

ETHANOL EFFECTS ON THE DEVELOPING FROG EYE. L.C. Towns, P.S. Sexton* and N.J. Uray, Kirkville College of Osteopathic Medicine, Kirksville, MO 63501.

Experiments were conducted to assess the effect of ethanol on morphological development of the optic tectum in bullfrog tadpoles. Groups of tadpoles were placed in a 1% (v/v) ethanol at two week intervals from the time of hatching to 42 days old. All animals were sacrificed at 6 months of age. In treated animals, a large continuous space develops between the ependymal and subependymal layers. When treatment was begun later at 2-3 weeks, the disruption at the ependymal-subependymal boundary is generally restricted to the areas of the posterior tectum which are currently developing. Previously developed portions of anterior tectum show less disruption. Similar spaces were present in untreated controls but they do not reach the size or continuity seen in ethanol-treated animals. These data indicate that ethanol treatment induces morphological alterations in proliferative areas of the optic tectum. This disruption may result from edema or alterations in microvasculature as previously reported in cerebellum. Supported by NIAA grant 07537.

476.17

ETHANOL-INDUCED ALTERATIONS IN THE MICROVASCULARITY OF THE TADPOLE CEREBELLUM. M. L. Uray and P. D. Sokoloff*, Department of Anatomy, Kirkville College of Osteopathic Medicine, Kirksville MO 63501.

The purpose of this study was to examine the effect of chronic ethanol exposure on the microvasculature of tadpole brains for morphological alterations which may be associated with the increased severity of alcohol withdrawal symptoms. Tadpoles were raised in 1% (v/v) ethanol after the animals were killed and the brains were prepared for light microscopy. Observations were made of the choroidal vessels and arachnoid vascularity, along with examination of the brain tissue for signs of extravasation. Our observations suggest that after chronic ethanol exposure the choroidal vessels of the tadpole plexus were more tortuous in the ethanol-treated animals than in control animals and 2) the average vessel diameter in ethanol-exposed animals (9.0±0.6 μm) was significantly (P<0.01) greater than in control animals (6.0±0.2 μm).

Furthermore, in the brain tissue of some of the ethanol-treated animals, blood cells were observed in the extracellular spaces. These findings support the notion that some of the morphological changes induced by chronic ethanol exposure observed in the developing tadpole brain, may be attributable to changes in the microvasculature, rather than altered neurogenesis. (Supported by NIAA grant 07537.)

477.1

HOMEBOX GENES EXPRESSED IN ADULT MOUSE CEREBELLUM. Robert E. Bullitt, Timea L. Banitez, Dept. of Pharmacology, University of Maryland School of Medicine, Baltimore, MD 21201.

Homebox genes encode one class of transcriptional regulatory proteins which may play a role in differentiation of brain neurons. Homebox genes are expressed in several populations of vertebrate neurons. In invertebrates the expression of specific homeobox genes is required for differentiation of certain neurons. Our interest is to identify homebox genes that are involved in the differentiation of specialized cells. We have focused on two classes of homedomain proteins. One class includes the murine Hot family of proteins which contains a highly conserved homedomain. The second class includes the POU/Homeo domain proteins which also contains an additional highly conserved POU domain. The polymerase chain reaction (PCR) has been used to amplify and clone cDNAs encoding members of these two classes of homedomain protein families that are expressed in the adult mouse cerebellum. Two sets of full codon degenerate oligonucleotide primers were synthesized for use in the PCR experiments. The first set of primers is based on two regions in the homedomain of the murine Hot protein. The second set of primers is based on two conserved regions in the POU/Homeo domain proteins, one is the homedomain and the second is the POU domain. The length of the sequence between the two primers provides an indication of the size of DNA fragment expected to be amplified by PCR. The template DNA used for PCR was cDNA synthesized from poly A+ RNA obtained from adult mouse cerebella. Using these two sets of primers we have been able to amplify DNA fragments of the appropriate size as observed on ethidium bromide stained agarose gels. The amplified DNA was cut from the gel and cloned into the pGEM7Z vector. Individual clones were selected and run sequencing reactions. The number of different homedomain sequence identified from the adult cerebellum. In situ hybridization on tissue sections of the cerebellum will be employed to characterize the cellular distribution of the expressed homebox genes.

477.2

IDENTIFICATION OF HBH-1, A HOMEODOMAIN-CONTAINING TRANSCRIPT FROM HUMAN PEDIATRIC BRAIN. D.L. Szaki, M. Mount, A. B. Wadhams*, P. D. Coleman, N. Thomas*, K. E. Rogers, Departments of Neurobiology and Anatomy and Dermatology, University of Rochester Medical Center, Rochester, N.Y. 14642, USA.

Homeobox proteins are thought to be involved in the differentiation and function of specialized cells. In order to determine which homeobox proteins are active during neuronal development we have designed a mixed oligonucleotide probe corresponding to amino acid positions 44-155 of the murine HBH-1 gene. Prehybridization with nonhomologous probes containing 11 through 21 weeks of age. (Supported by NIH grant AG 00107 and ARDRA PRG 89-120)

To investigate developmental changes occurring in central nervous system and neural crest, a series of chick embryos were immunostained with several monoclonal antibodies. HNK-1 immunoreactivity appeared in rhombomeres (r)3 and r5 around stage 12. This immunoreactivity was the most extensive at stage 15, when r2 and r4 were not stained. This alternating pattern is similar to the Krox-20 gene expression in the mouse embryo (Wilkinson et al., 1989a). At levels of r2 and r4, neural crest cells were attached to the hindbrain. An accumulation of neurons was observed in these rhombomeres near this attachment. The above observations seem to suggest that the alternate HNK-1 immunoreactivity in rhombomeres might be related to pair-rule-like segmentation of the nervous system in concordance with the hindbrain. This phenomenon is the first example of a phenotypic differentiation of CNS seemingly related to homeobox-containing gene expression.

477.4 POSSIBLE ROLE OF BASIC FIBROBLAST GROWTH FACTOR IN THE DEVELOPING AND REGENERATING RETINA OF GOLDFISH. Pamela A. Raymond and Linda K. Barthel. Univ. Michigan, Dept. Anatomy & Cell Biology, Ann Arbor, MI 48109. Antibodies to basic FGF isolated from bovine brain bind to various specific cells or laminin in the larval and adult fish retina and the most intense immunoreactivity (blood vessels excepted) is in horizontal cells (HCs) and outer plexiform layer (OPL). This observation is intriguing since in this retina, rod photoreceptors are added continuously through mitotic divisions of rod precursor cells located along the edge of the outer nuclear layer adjacent to the OPL. Double label studies with GABA antibodies (which stain the predominant type of HC in fish) suggest that some but not all of the FGF immunoreactivity co-localizes with GABAergic HCs. It has been proposed that GABAergic HCs may play an organizing role during development of photoreceptors and outer retina. We have tested the hypothesis that FGF may be involved in this function by inserting ethylene vinyl acetate pellets containing FGF antibodies into the eyes of normal fish and those with regenerating retinas. Preliminary results indicate that the cytarchitecture of developing retina may be affected by FGF antibodies: laminar organization is disrupted and some neurons are in ectopic positions. Supported by NIH EY04318.


The mammalian striatum can be divided into the patch and matrix compartments based on the distributions of neurochemical markers and anatomical connectivity and based on striatal development. The patch and matrix compartment neurons are born at different times during rat development, with majority of patch neurons becoming postmitotic (embryonic E14) before the majority of matrix neurons (E18-20). Cell lineage studies suggest that the striatal compartments develop from separate pools of progenitor cells. The neurons of the patch compartment are adhesive to one another, as shown by their ability to selectively aggregate in culture. In order to investigate within compartment adhesion, we asked if adhesion differed amongst neurons of the patch compartment. Maternal injections of one of H2-thymidine or bromodeoxyuridine were made on E13 and were followed in the same pregnant rats by injections of the alternate label on E15. In order to mark new postmitotic patch neurons at early and later times of patch neurogenesis. Animals sacrificed at postnatal day 30 revealed that early born neurons are preferentially located towards the centers of patches, whereas later born patch neurons are preferentially distributed towards the peripheries of the patches. Thus, in addition to a previously described qualitative difference in the self-adhesiveness of striatal patch neurons versus the lack of such a self-adhesiveness among striatal matrix neurons, there is also a difference in adhesiveness among neurons of the patch compartment. The increased adhesiveness of the earlier born patch neuron may serve to nucleate the patches during striatal development.

477.6 POSTMITOTIC STRIATAL PATCH AND MATRIX NEURONS INTERMIX BEFORE COMPARTMENTS ARE FORMED. L.A. Krushel, G. Fischb and D. van der Kooi. Dept. of Anatomy, Univ. of Toronto, Toronto, Ont. Canada, M5S 1A8.

Neurons of the patch compartment in the rat striatum become postmitotic earlier in neurogenesis than neurons of the matrix compartment. The previously demonstrated selective adhesion of patch cells to one another may be an important component in the development of striatal compartmentalization. We examined both in vivo and in vitro whether the selective adhesion of patch cells is expressed before or after the migration of the matrix neurons into the striatum, in vivo, patch neurons were labeled by a fluorescent retrograde tracer (true blue) injected into the substantia nigra on embryonic (E) 11 (E) (1) by an electrophoretic injection of the newborn (E14) cells to one newly formed out of the striatum. Matrix neurons were labeled with a maternal injection of bromodeoxyuridine (Brdu - a birthrate marker) on E18. On E20 true blue labeled patch cells were found intermingled with the Brdu labeled matrix neurons in the medial striatum, however further laterally small clusters of labeled patch cells were present. By postnatal day 2 there was a complete segregation of the clusters of labeled patch neurons from the matrix neurons in the striatum. This process was also modeled in vitro. The patch and matrix compartments were labeled in vivo with different birthdate markers (H2-thymidine or Brdu) on E13 and E18, respectively. On E20 the striatal tissue was removed, disassociated and reaggregated in suspension cultures. After 1 day in vitro, labeled patch and matrix cells were randomly intermixed within the reaggregates. Examination of the cultures after 5.5 and 4 days in vitro revealed clumping of the labeled patch cells towards the centers of the reaggregates. Over this same period, the labeled matrix neurons did not clump and were dispersed towards the periphery of the reaggregates. Thus, the selective adhesiveness of patch neurons is either expressed after the later born matrix neurons begin to migrate into the striatum or is unable to overcome the force of the massive migration of matrix neurons. We conclude there is a migratory phase when committed patch and matrix neurons intermix before expressing their adhesive phenotype in the postmitotic striatum.

TRANSPANTATION: RECEPTOR EXPRESSION


Rat cerebral cortex contains numerous neurotransmitters and receptors. Of particular interest is the neurotransmitter gabaergic/gatin releasing peptide (BN/GRF) whose receptors develop on fetal neocortex transplants into the adult striatum (Gietz et al., Neurosci. Lett. 79:97 (1987)). BN/GRF functions as a potent safety and grooming agent in the rat CNS and stimulates the growth of certain neuropeptides in the striatum. The binding properties of these fetal cortex transplants were investigated using in vitro autoradiographic techniques. High densities of (3H)TRITIBN grains developed in fetal cortex transplant into the host ventricle or adult cortex but not on cerebellar or superior cerebral gyrilla transplant. These results indicate that the development of BN/GRF receptors is a function of the transplant and not the host tissue. The autoradiographic grains, which were analyzed on an Amersham RAS 3000 densitometer, developed 3 weeks after transplantation in the host tissue and were retained for at least 3 months. The amount bound was a function of (3H)TRIBN concentration. The rate of radioligand unbound was calculated for (3H)tritiated (TRIB)BN bound with high affinity (KD = 4 nmol/L) to a single class of sites (Bmax = 110 fmol/mg protein). GRF and GRF-2 but not GRF-1 inhibited specific (3H)TRIBN binding with high affinity (IC50 = 2.5 x 10-10 M) respectively. Also, BN receptor antagonists such as (Phe)4-LES(9), LES(9) and (D-Arg, D-Pro, D-Trp, LES) substances C competed for the (3H)TRIB-binding sites (IC50 = 100 and 900 nM respectively). Because BN/GRF receptors develop on fetal cortex transplants, they may facilitate the growth of the graft tissue. Supported by NIH grant NS-17468 and NSF grant HR-15332.

478.2 CHANGES IN STRIATAL DOPAMINE RECEPTOR BINDING FOLLOWING ADRENAL MEDULLA GRAFTS. E.J. Curran and J.B. Becker. Neuroscience Program and Department of Psychology, The University of Michigan, Ann Arbor, MI 48109.

We have previously shown that intraventricular adrenal medulla (AM) grafts transplanted adjacent to the dopamine (DA) denervated striatum produce a decrease in rat activity induced by either amphetamine (AMPH) or apomorphine (AP). Experiments using quantitative autoradiography were conducted to determine whether changes in striatal DA receptors are associated with this behavioral recovery. Adult rats with unilateral substantia nigra lesions were tested for AM- and AMPH- induced rotational behavior prior to and after receiving intraventricular AM grafts. Ten to twenty days following the last drug treatment, the rats were decapitated, the brains were rapidly removed and frozen. D2 and D1 receptors were determined by : binding to D1 receptors or [H]spiperone binding to D2 receptors. Preliminary experiments revealed that D2 receptor binding increased in the DA denervated striatum. Behaviorally effective AM grafts attenuated this increase. In contrast, no apparent change was seen in D1 receptor binding in the DA denervated animals. (Supported by grants NS 231475 & UM 363145.)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
478.3
BEHAVIORAL RECOVERY ASSESSED WITH D1 AND D2 DOPAMINE AGONISTS AFTER VENTRAL MESENCEPHALIC GRAFTS INTO DOPAMINE DENERVATED RAT STRIATUM. J.B. BECKER, J. B. McFARLAND. 1Psychology Dept & Neuroscience Prgm, Univ. Michigan, Ann Arbor, MI 48104; 2Anatomy and Neurobiology, UVM College of Medicine, Burlington, VT 05405.

Simulation of both D1 and D2 dopamine (DA) receptor subtypes synergistically activates behavior mediated by the intact striatum. Following striatal DA receptor damage, locomotor activity is thought to be unaltered; administration of either a D1 or a D2 DA receptor agonist will induce rotational behavior (J. Pharm. Exp. Ther., 247:160, 1988). Previous experiments have demonstrated that turning behavior induced by either apomorphine (AP0) or amphetamines (AMPH) is decreased after grafts of fetal mesencephalic tissue into the DA denervated striatum. Since AMPH and AP0 will activate both DA receptor subtypes, this experiment was conducted to determine whether behavioral recovery is associated with a single DA receptor population.

Female rats with a unilateral 6-OHDA lesion of the substantia nigra underwent behavioral testing before and 2-3 months after intrastriatal grafts of fetal mesencephalic tissue. Animals were tested with the full D2 agonist SKF26195 (0.5 mg/kg); the D2 agonist, LY171555 (0.01 mg/kg); and AMPH (3.0 mg/kg). Survival of DA containing grafted cells was confirmed autoradiographically 4 months post-graft with catecholamine fluorescence. Preliminary data indicate that the transplantation of DA containing fetal brain tissue can produce decreases in rotational behavior induced by SKF26195 (range = 10% to 75%), LY171555 (15% to 90%) and AMPH (15% to 95%). We conclude that behavioral recovery induced by fetal mesencephalic tissue in Parkinson's Disease involves compensatory responses by both D1 and D2 DA receptor subtypes.

[Supported by USPHS NS 22157 (UBB) and NS 30507 (MAA)].

478.4
D, AND D2 RECEPTOR MORPHOLOGY AFTER PLACEMENT OF VENTRAL MESENCEPHALIC GRAFTS IN DOPAMINE DEPLETED RAT STRIATUM. M.A. ARTIENS, J.B. BECKER, J.D. McVIEHLE, & D.R. SHIELDS. 1Psychology Dept & Neuroscience Prgm, Univ. Michigan, Ann Arbor, MI 48104; 2Anatomy and Neurobiology, UVM College of Medicine, Burlington, VT 05405.

There are two distinct dopamine (DA) receptor subtypes. The D2 receptor stimulates adenyl cyclase. Striatal D2 binding sites are found morphologically associated with neurons containing CAMPR immunoreactivity. Stimulation of striatal D2 receptors inhibits cAMP production. Striatal DA denervation produces specific receptor changes; the association between D2 binding sites and CAMPR immunoreactivity is abolished and there is an increase in the number of D2 receptors. This study was initiated to determine whether transplanting fetal mesencephalon into the DA-denervated striatum could induce recovery of these morphochemical indices of DA receptor function.

Female rats with a unilateral 6-OHDA nigral lesion, underwent behavioral testing (results reported separately) before, and 2-3 months after transplantation. Cellular distributions of the DA receptors were examined after 4 months. The striatal distribution of both DA receptor subtypes was determined by application of rhodamine derivatized antagonist ligands (KAS 88:8570, 1990). The morphochemical association pattern of D1 receptors was assessed by in utro autoradiography with immunohistochemical staining of CAMPR (Brain Res. 443:204, 1988). D2 receptor distribution was further characterized by specific antibodies generated against portions of the peptide sequence for the native protein (FASEB J. 4:601, 1990). Preliminary data suggests that the transplantation of DA-containing fetal brain tissue provides a sufficient milieu for recovery of the DA receptor distribution. This data suggest a morphological basis for behavioral improvements induced by grafts of fetal ventral mesencephalon in this animal model of Parkinson's Disease.

[Supported by USPHS NS 23079 (MAA) and NS 22157 (UBB)].

478.5
AUTORADIOGRAPHIC STUDY OF D1 AND D2 RECEPTORS IN THE DIFFERENT REGIONS OF THE STRIATUM AFTER CHRONIC TREATMENT WITH L-DOPA IN 6-OHDA LESIONED RATS WITH AND WITHOUT GRAFTS. D. DI Paolo, M. DiPaolo, J.B. McFARLAND. 1Psychology Dept & Neuroscience Prgm, Univ. Laval, Quebec, (QC), CANADA G1K 7P4.

We have previously shown that fetal dopamine transplants in the striatum prevent supersensitivity of the DA receptors induced by denervation itself or by repeated administration of L-Dopa (Gaudin et al., Soc. Neurosci. Abstr., 15:1335, 1989). In the present work, we have studied the effects of a similar striatal graft on dopamine D1- and D2-receptors in the targets structures of the striatum. Two groups of rats were prepared with a unilateral lesion of the nigro-striatal DA pathway with 6-OHDA. One group received a graft of 1.5 X10⁶ midbrain cells. Each group of animals received 14 injections of L-Dopa, 2 mg for the first 4лице, 2 mg for the last 7 injections. The animals were sacrificed for autoradiographic study of the D1[H]-SCH 23390 and D2 [H]-Stereopine) receptors. The results show a decreased density of D1 receptors in the GP (77%), EP (58%) and SN (52%) in the SN of the grafted side compared to the lesioned ungrafted side of the second group. On the other hand, an increased density of D2 receptors was observed in the GP of the grafted side (25%) and surprisingly of intact side of grafted side. The density of D2 dopaminergic receptors was decreased on the lesioned and grafted side (57%). Our results show that a fetal nigral graft can modulate the changes of D1 and D2 receptors caused by dopaminergic denervation and by chronic levodopa treatment in striatal target structures. The modifications of dopaminergic receptors in the different regions of striatum of grafted animals may also explain the absence behavioral supersensitivity (Gaudin et al., Br. Res. 506: 1, 166-168, 1990) after chronic treatment with L-Dopa.

Supported by MRC of CANADA.

478.7

Three experimental groups (control group, lesion group and lesion + graft group) of rats were used for this study. The mesencephalic system of the animals was unilaterally destroyed by 6-OHDA injected into the medial forebrain bundle. Three weeks after the lesion, a cell population containing embryonic dopaminergic neurons was implanted into the denervated striatum. Six months after the graft animals were sacrificed. Autoradiographic studies carried out in parallel from consecutive sections of the same animals evidenced an increase of D2 dopamine (DA) receptors densities which was reversed by the implantation of embryonic dopaminergic neurons. In the present study, using autoradiographic techniques, we examined whether levels of mRNA coding for D2 DA receptors vary in the same way as those of the D2 receptor protein itself. [32P]-labeled oligonucleotide derived from the coding region of the rat DA D2 receptor gene was used as a probe to localize in the rat brain sections the cells containing the mRNA coding for this receptor. The distribution of mRNA was comparable to that of the dopamine D2 receptor binding sites as visualized by autoradiography of [3H]-Stereopine or [3H]-Raclopride. The lateral part of the striatum presented high mRNA content comparatively to the other subregions of this structure. In the denervated striatum, the mRNA levels in the lateral striatum as described for the supersensitive D2 receptors, after implantation of embryonic dopamine neurons the expression of mRNA for this receptor was normalized. These results provide evidence that functional recovery of DA neurons graft is mediated at least in part through the modulation of the genetic expression of the D2 receptor.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
478.1
WEAK ELECTRICAL FIELDS SPEED UP REGENERATION OF MOTEURONUS IN THE CRUSHED SCARRIC NERVE OF NINE MONTH OLD RATS. J.E. Campbell and B. Pomeranc.

Our lab has previously reported that motoneuron regeneration is accelerated when treated with weak electrical fields in rat and rabbit sciatic nerve of 10 week male rats (McDevitt, L. et al., Brain Res. 416:308, 1987). The present study was conducted to determine whether: 1. the speed of nerve regeneration is slowed with age, and 2. if so, will 10 uA direct current stimulation accelerate regeneration in the old animals with crushed nerves?

Seventeen 10 week male Wistar rats underwent a crush of the right sciatic nerve. This group was obtained Young Crush (YC). In 10 rats the crush was made at 9 mo of age (Old Crush = OC). Seventeen 9 mo rats received a crush and had battery driven weak electric devices implanted in the neck region with the cathode distal wick passing by the site of the lesion (Old Crush Cathodal Distal = OCCD). These implants drove a current of 10 uA by the nerve through the recovery period. Fifteen 9 mo rats underwent a crush and were implanted with sham devices (Old Crush Sham = OCS).

On post-operative day 25, EMGs were recorded by stimulating the sciatic nerve transcutaneously via micro-alligator clips while recording pins were inserted in one of 12 identified sites in the rat's right foot. 70.8% of all sites were innervated in the YC while only 26.7% of the sites were innervated in the OC suggesting that regenerative speed slows with age. Also, the OCCD had 68.8% of all sites innervated, while the OCS had only 18.9%, indicating that DC fields speed up the rate of regeneration.

478.2
QUESTIONING THE PROPOSED ENHANCEMENT OF PERIPHERAL NERVE REGENERATION BY APPLIED ELECTRIC FIELDS. M. E. McQuinn.

Several papers have been published reporting an enhancement of the rate of peripheral nerve regeneration in rat sciatic nerve due to the application of weak DC electric fields. Having been unable to demonstrate any effect of such fields on peripheral nerve regeneration in guinea pigs, we undertook to reproduce two of the published positive reports. An experiment by Politis, Zanata, and Andrus (J. of Trauma 29:665-673, 1988) consisted of placing a transection and anastomosis site within a silicone tube with electrodes at either end. An unregulated current source passed 1.5 mA through the tube for 12 days with a resulting 4 fold increase over controls in the number of neurofilament positive profiles found 14 mm distal to the anastomotic site. Our repeat of this experiment showed that the devices only passed 0.23 uA, most of which follows the low resistance pathway around the outside of the tube rather than going through it. At the time and distance indicated, I found no difference in the number of neurofilament positive profiles, or myelinated or unmyelinated fiber density.

Another paper (Ronchi et al. Exp. Neurology 99:229-232, 1987) reported a 5.5 fold increase in the number of myelinated fibers regenerating through a silicone tube (across a 5mm gap) when a cathode delivering 10 mA was located in the center of the tube. A repeat of this experiment with a larger sample size than originally used demonstrated no effect of the applied fields.

From these experiments it is apparent that the effects of applied fields on peripheral nerve regeneration is still open to question, and should remain so until a robust, repeatable effect can be demonstrated by several labs.

Supported by NIH grant NS 26251

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

479.3

Studies have been done that cathodally-directed DC. stimulation of damaged mammalian peripheral nerve results in an increased rate of regeneration toward target muscles. Constant DC. current was regulated to 10 microamperes, 1 c. or an I.C. at two voltage levels in the elongated PNS. Rat sciatic nerves were transected and then frozen with dry ice, and a galvanic nerve cuff was placed over the lesion site with cathode located 10 mm distal and anode located 10 mm proximal. The electrodes delivered A) either 1.4 or 14uA at 1.4v limited by a resistor or B) delivered 1.4 or 14uA at 9v limited by a resistor. Animals were allowed to survive for 6 to 15 days post lesion/treatment. Frozen serial sections of the nerve were reacted with fluorescent antibody to nervefilament protein. Results at 8 days showed that the electrodes at 1.4 and 14uA (at 1.4v) whose current was limited by a resistor demonstrated equivalent numbers of axons, yet contained significantly more axons than the I.C. regulated group animals, whoseaxon counts were equivalent in that group. At 15 days, theaxon counts were elevated by about 205 in all groups, but no statistical differences were found between groups.

479.4
REGENERATION OF DORSAL ROOT AXONS IS INFLUENCED BY INTERCELLULAR REACTIONS IN THE DORSAL ROOT GANGLION. X. Lu and P.M. Richardson. Montreal General Hospital and McGill University, Montreal, Canada B3A 1A4.

Do cellular interactions within the dorsal root ganglion contribute to the regenerative propensity induced in sensory neurons by peripheral nerve injury? To study this question, axonal regeneration was assayed in rat L4 dorsal spinal roots following a variety of interventions designed to inhibit or enhance such regeneration. Counts of thinly myelinated fibers 17 days after crush injury were increased by injection into the ganglion of isogogenous macrophages or the inflammatory agent, endotoxin. Intrathecal infusion of mitomycin C did not significantly retard the relatively vigorous dorsal root regeneration induced by cutting of the sciatic nerve. As assessed by thymidine radioautography, this infusion of mitomycin C did reduce the proliferation of satellite cells that normally follows sciatic nerve transection. These observations raise the possibility that perikaryal regenerative responses after nerve injury are not directly induced by axonal interruption but indirectly by secondary reactions of satellite glial cells surrounding the nerve cell body. However, satellite cell proliferation is not essential for this stimulation.

479.5
TRANSDIFFERENTIATION OF XENOPUS RETINAL PIGMENT EPITHELIAL CELLS INTO GLIAL-LIKE CELLS IN CULTURE. D.S. Sakaguchi. Dept. Biol. B-022, UCSD, La Jolla, CA 92039

The retinal pigment epithelium (RPE) of amphibians can transdifferentiate into neurons and lens cells in culture. I report here that the RPE, in addition, is capable of transdifferentiating into glial-like cells. For these studies the posterior two-thirds of larval Xenopus (pluteus; 4400) RPE were isolated and placed in primary culture. After 1 week RPE cells were harvested and replated under limiting dilution conditions. Using this approach several clonal lines were established from single RPE cells, 2 of which are discussed here. To identify different cell types which transdifferentiated in culture a panel of cell-type specific antibodies was used. Using these an RPE specific serum (XR1), and not were labeled by glial cell markers (anti-vimentin, anti-GAP43 or RS mAb). However, after 2 months in culture, RPE clonal cell line inhibition and were no longer immunoreactive to the RPE specific mAb. In addition, the RPE derived clonal lines were now labeled by the glial cell markers. These cell lines were then assayed for their ability to promote neurite outgrowth from embryonic retinal explants. Although collagen alone was relatively ineffective at promoting neurite outgrowth, collagen substrates directly conditioned by the RPE derived cell lines were capable of promoting neurite outgrowth following chemical extraction with Triton X-100. This ability of RPE cell line derived extracellular matrices to support neurite outgrowth is a feature shared with the XR1 cell line, a Xenopus glial cell line derived from the retinal neuroepithelium.

479.6

ACTH/MSH peptides accelerate peroneal and sciatic nerve regeneration in the adult rat, improving qualitative and quantitative measures of the time course. In the neonatal period, the role of MSH and ACTH 4-10 on the regeneration of the developing motor system after trauma. Two-day old Sprague-Dawley rats are subjected to sciatic nerve crush with a #5 forceps. Positive results demonstrate that peptides (10 µg/kg/88) is from time of surgery continuing 6 or 8 days. At days 7 and 15, the EDL muscles are fixed in situ and the NMJ are prepared for light microscopy with a modified silver-hematoxylin stain. Peptide treatment (10 µg/kg/88) is significantly increased when added to the diet 1 day after surgery. Peptides significantly increase innervation density, while MSH also increases area and perimeter as compared to saline controls. Muscle fiber diameter is decreased with both peptides, significantly by ACTH 4-10 only. At day 15, both peptides increase NMJ area, perimeter and fiber diameter; interior branching is comparable in all groups. These results continue to support the significant role of ACTH in peripheral nerve regeneration.

147.7 DIPLOTE MORPHOLOGY AND WALKING TESTS ARE ENHANCED BY
BIM 22015 (ACTH 4-10 ANALOG) DURING PERIPHERAL NERVE
REGENERATION. T.S. Lee, Z.J. Lee, R. Foster, and F.L. Strand.
Department of Biomedical Engineering for Neurological Disorders.
New York University Medical Center for Neurological Sciences.
Washington Square, New York, New York 10030.
Spinal-Damaged (175-200g) rats are subjected to peroneal nerve crush
or 8% choral hydrate anesthesia. A #5 forceps is used, resulting in a
1 mm wide lesion. Starting at the time of the surgery, an ACTH 4-10 analog
BIM 22015, 0.1mg/kg, was given 3 or 5 times a week for 1 to 4 weeks after
nerve crush. BIM 22015 treated animals show a significant increase in
endplate diameter and nerve terminal branching. At 7 days post-surgical
nerve treatment increases endplate area, internal branching and muscle
fiber diameter. The waking test consists of having the animals walk up an inclined
pathway, after having their feet dipped in non-toxic ink. The resulting
footprints permit the measurement of toesprints, print length and the
situation of the hindlimb Function Index. The delineated foot is used as a control
for the lesioned foot and parameters are compared to saline
injected controls. BIM 22015-treated rats show enhancement of recovery
as determined by these parameters. Several ACTH peptide fragments (ACTH
4-10 and a ACTH 4-9 analog, Org 2768) improve peripheral nerve regeneration
and endplate morphology following nerve crush. This ACTH 4-10 analog, BIM 22015, can now be included in this family of neurotrophic

147.9 THE EFFECT OF LOCAL ADMINISTRATION OF EXTRACELLULAR MA-
TILE AND NERVE GROWTH FACTORS ON FUNCTIONAL OUTCOME FOLLOW-
ING NEONATAL THORACIC SPINAL CORD TRANSSECTION IN THE
RAT. B.W. Walters, R.B. McCaslin*, and T.E. Melin. Div. of
Neurosurgery, Univ. of North Carolina at Chapel Hill, NC 27599-7060.
Sprague-Dawley rats have been studied in a chronic spinal injury model designed to demonstrate the eff-
fects of local application of extracellular matrix with or without additional growth factors on functional
outcome. An open procedure was performed under methoxyflu-
rene anesthesia with an operating microscope in the mid-
 thoracic region within 36 hours of birth. 13 had laminecto-
my alone, 1 had intradural transection and one segment myelotomy (TRANS). 9 had myelotomies with implantation of approx. 7 ml of matrigel (ECM), and 5 had a control consisting of approx. 3.5 ml of 2.5% HGF (WGF). The animals were evaluated by the combined behav-
ioral score of Walkathl (Exp. Neurol. 88:123, 1985) 5 times
weekly for 15 weeks in blinded fashion. The LAMT animals performed significantly better (p<0.0003, ANOVA)
than all other groups on weeks 3-15. From 5-10 weeks ECM animals outperformed TRANS animals (p<0.05 at 5 & 6,
p<0.01 at 6). In contrast, WGF animals performed the same as TRANS animals through week 6 and then performed worse poorly (p<0.05 to <0.001). The neuropathological cor-
relates of these results are being investigated. Sup-
ported by the American Paralysis Association.

147.11 ORGANIZATIONAL PATTERNS OF REGENERATING NERVE FIBERS AFTER
SPINAL CORD COMPRESSION INJURY. G.F. Barrett, R. Reaps*, and
Spinal cord lesions in untreated compression injury of
rat spinal cords reveal necrosis that begins a few days
post injury and eventually extends rostrally through 3-4
spinal segments. In such lesions neurite outgrowth is
severely restricted or absent. However, similar injury in
lipopolysaccharide treated rats (0.1mg/liv thrice
daily for 8 weeks) is followed by less severe reactive
changes, regeneration and gliosis, and the formation of
cellular bridges between the caudal and rostral edges of
the lesion. Silver and immunocytochemical staining subse-
quently reveal neuritic regeneration associated with the
bridges. Electron microscopy further reveals tubular
shaped bridges separating regenerating neurites, and
swelling Schwann cells, occasional macrophages and astrogli-a,
blood vessels, and connective tissue. The time course of
appearance and the amount of new cell growth in the
bridges and Schwann cells in relation to the neurites resembles
conditions observed in peripheral nerve regeneration. Thus the
cellular substratum in peripheral nerves is an effective
bedway of trophic support and chemoattractant guidance for
regenerating peripheral or central neurites, the cellular
bridges could afford a means for central regeneration. To
test this we have injected unconjugated type V NGF retrogradely to the
lesion site of saline or LPS treated rats and examined spinal cord at 2 weeks post injury to the lesion.
Preliminary results showed that in the LPS treated rats there were very few in spinal motilial and
dorsal root ganglia, indicating that both central and
peripheral regenerating neurites are present in the lesion.

147.8 SEX DIFFERENCES IN REGENERATION AFFECT ACH RECEPTOR
CONCENTRATION AND MOTOR RECOVERY. J. Kimm and F.L. Strand.
Six striolocchi have recently identified a newborn-sensitive nerve, the external digitorum longus (EDL), Segarra, A., Bölsch, B.,
concentration and recovery in the EDL, after nerve crush in castrated and normal rats. Male

and female rats were divided into 3 groups: sham-crushed; nerve crushed; and

castrated, nerve crushed. Under anesthesia, the peroneal nerve was crushed at the site of innervation. For the AChR assay, males were castrated using an
LHRH antagonist (BIM-23006, Biomasure, Inc., 5mg/kg.s.c.). Females were surgically castrated. AChR concentration was then measured by radio-
immune assay with [3H]-alpha-bungarotoxin. Motor tests were done on rats used for the AChR assay, in a separate trial where some males underwent nerve crush and surgical
castration during the peripheral stage. Day 11. Distance: 1.5 (DO1.5) was measured by the method described by Bain (Plastic & Reconstr. Surg., 1985,
83:129). Nerve crush increases AChR concentration, especially in castrated
animals. Under anesthesia, the peroneal nerve was crushed at the site of innervation. For the AChR assay, males were
shown higher AChR concentration than females. This difference is not seen in rats with normal sex levels. However, when relating this difference to DO1.5, only castrated
males had a larger too small than their female counterparts. In the second trial, no such motor difference
was noted for any nerve-crushed groups. In fact, when comparing absolute DO1.5, print length, and personal functional index ratios, these males
recovered less than females. Crushing the peroneal nerve, we found that testicular levels have not yet peaked to adult levels restricts the positive
neurotrophic pattern of recovery from occurring. (Supported by NIH training grant# MH18862-03)
479. 13
FLASHWOUND ACTIVATOR AND SCHWANN CELL PLASTICITY IN PERIPHERAL NERVE REGENERATION. N. Kalderon. The Rockefeller University, New York, NY 10017.

Flashwound activator (PA) is a key enzyme controlling extracellular proteolytic activities. Mannamalian cells produce 2 molecular forms of PA: urokinase type (PAu) and tissue type (PAT) at least 2 fibrinolytic inhibitors (PAI), PAI-1 and PAI-2. The PA in concert with PAIs regulates cell migration and ECM degradation in plasticity events. In differentiated Schwann cells express high PA activity levels (Kalderon, 1984, PMAS; B: 7216) primarily of the u-PA type (Kalderon et al. in Regulation of Extracellular Fibrolysis in Nervous System Development & Disease, Plenum). We are examining whether these plasticity properties of Schwann cells are due to their presence in support of nerve regeneration; the effects of protease inhibitors on rat sciatic nerve regeneration through a 10mm sciatic nerve chamber are being studied (Kalderon et al., 1987, SM Acta 13:1208). The nerve stumps are nutured to a silicone tube which is filled with a physiological salt solution. The inhibitors are injected into the chamber at 7 days post surgery when a massive cell migration ensues, and their effect on nerve regeneration (Schwann cell migration and axonal growth) was examined a week later. Depending on PAI here, PAI-1 (jug/ml) and aprotinin impaired cell migration at 522 and 48T, respectively, as compared with control samples; accordingly, axonal regrowth was delayed. These results and previous data (Kalderon et al., 1987) suggest that PA and plasmin activities are elaborated by the Schwann cells for migration and are essential for successful nerve regeneration.

479. 15

We have previously demonstrated that testosterone propionate (TP) accelerates functional recovery from facial paralysis induced by facial nerve injury in castrated male hamsters. In this study, we tested the hypothesis that the effects of TP on nerve regeneration occur at the level of the neuron and through a mechanism involving priming of the genome. Analysis of the effects of TP on rRNA levels was accomplished using in situ hybridization and rRNA probes. Male hamsters were received as castrates from the supplier. Following anesthetization, the animals were each subjected to a right facial axotomy, with the left side serving as internal control. One-half the animals immediately received subcutaneous implants of TP (TP + AX), with the others sham-implanted (AX only). Postoperative (P) tests were done ranging from 30 to 14 T. At the appropriate PO time, the animals were sacrificed by ether overdose and decapitation, and the brains rapidly blocked and frozen. Standard in situ hybridization techniques were used. Procedures were done on 8-um, postfixed sections using a 1H-28S rRNA probe. Computerized image analysis was employed for data collection. For both TP + AX, and AX only groups, the rRNA levels were expressed as a percentage of left side control. The results indicate that TP accelerates the initial rRNA increases observed with AX alone, as well as producing a dramatic effect on the magnitude of the response. Supported by an SCRF grant (KJJ).

479. 16
RENEWAL OF MEDIAL CORTEX BY CHOLINERGIC PROJECTIONS FROM BASAL FOREBRAIN: AN IN VIVO MODEL TO TEST HYPOTHESES ABOUT REGENERATION WITH GROWTH FACTORS. T.W. Farris and L.L. Bushberg. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563.

To determine further the regenerative effects of putative growth-promoting factors on cholinergic projections under various conditions (previously assessed for GM1 ganglioside and estradiol), we administered thymoxine (T4, 2.5 mg/kg, ip) or saline daily for 14, 30 or 60 days to 8-week-old female rats with unilateral knife cuts of the trigeminal cholinergic pathway arising from the cholinergic basal nuclear complex (CBNC) and projecting to cingulate and occipital medial cortices. Brain tissue was processed histochemically for acetylcholinesterase (AChE); immunochemical for choline acetyltransferase (ChAT), dopamine a hydroxylase (DBH) and nerve growth factor receptor (NGF); and for Nissl substance. Computerized densitometry performed by light microscopy showed, in both groups, the expected accumulation and depletion of all markers proximal and distal, respectively, to the cortical cut. However, the rate of recovery from distal depletion was significantly greater in T4-treated rats. Flouro-gold placed distal to the cut revealed cell bodies in the vertical limb of the diagonal band and magnocellular preoptic nucleus. It was possible to directly assess axonal regeneration by observing fibers crossing the cut, as occurred in a few cases in 30 and 60 of T4 rats. These data (1) support recent findings from this laboratory that CBNC neurons are sensitive to thyroid hormones and (2) demonstrate that medial pathway axotomy is a useful model for determining whether factors known to possess growth-promoting activity in vitro induce axonal regeneration in vivo. [Support: NIH NS 10926]

THE AGING PROCESS: CELL BIOLOGY, MORPHOMETRY, OTHER

480. 1
MAP-2 AND TUBULIN DEGRADATION BY CATHESPIN D. J.M. Lipton, G.V.W. Johnson, and J.N. Whitaker. Dept. of Neurology, Psychiatry, Cell Biology Univ. of Alabama and the VA Medical Center, Birmingham, AL 35294.

The proteolysis required for the normal turnover of neuronal cytoskeletal proteins, such as MAP-2 and tubulin is poorly understood. Cathespind (EC 3.4.23.5), an aspartyl endopeptidase present in lysosomes and endosomes is abundant in many neurons. Specifically, in the in vitro degradation of MAP-2 and tubulin by cathepsin D was measured at various enzyme-to-substrate ratios and pH conditions using quantitative immunoblot techniques. Cathespind showed a much greater sensitivity to the pH conditions. At pH 3.5 and a 1:20 enzyme-to-substrate ratio, MAP-2 was totally degraded after 20 min, whereas tubulin under the same conditions was only degraded after 20 min, and a small additional substrate loss even after 60 min. The pH dependence of the cathepsin D-induced hydrolysis of MAP-2 and tubulin was also very different. At pH values between 3.0 and 5.0 the rate of degradation for the MAP-2 was 2.8 fold higher than for tubulin. Hydrolysis was not significant at pH 5.5, whereas there was significant degradation at pH 6.0 only a small amount of MAP-2 was degraded even after 60 min. In contrast, the hydrolysis of tubulin by cathepsin D was optimal at pH 4.5. At pH values of 3.0, 3.5, 5.0 and 5.5, tubulin was degraded by cathepsin D, but at significantly decreased rates, at pH 6.0 there was no significant hydrolysis of tubulin. These results demonstrate that MAP-2 and tubulin are equally susceptible to degradation by cathepsin D. These data also indicate potential for rapid degradation of MAP-2 by cathepsin D either in lysosomes, or in endosomes of the neuron. It appears unlikely that tubulin is a major substrate for cathepsin D. An understanding of these degradation processes may have relevance in neurodegenerative diseases such as Alzheimer's disease, where abnormal processing of cytoskeletal proteins may be a cental making of the disease. Supported by NIH grants NS27538 and AG06569, the Alzheimer's Association/Mary Sue Glover Memorial Pilot Research Grant and the Research Program of the Veterans Administration.

480. 2
AGE RELATED CHANGES IN c-fos EXPRESSION IN MICE. A. D'Orosta, R.L. Boose, C.R. Brese, R.L. Boyd and W.E. Sonntag. Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

c-fos belongs to a class of genes that encode for nuclear proteins presumed to participate in the regulation of cellular growth and differentiation. A "third messenger" role is postulated in signal transduction systems and its expression has been shown to be induced by a variety of exogenous and endogenous stimuli. Recent data indicate that the expression of the gene for nuclear c-fos may be regulated by transcriptional or posttranscriptional mechanisms. (J. Seashadi et al., Science 247, 1980) which suggest a possible involvement in the mechanism of aging as well. This experiment examines the differential expression of c-fos in various brain areas after a single electroconvulsive shock in four, eleven and twenty-five month old mice. Mice were given an acute electroconvulsive shock (90V for 0.2s), without anesthesia, through earclip electrodes. All animals exhibited generalized tonic-clonic seizures lasting 30-40s. At one hour after the seizure, animals were anesthetized and perfused with 4% paraformaldehyde. The brains were Vibratome-sectioned (50 microns) and examined using c-fos antibody (11:1000;CRB), directed against a conserved region of both mouse and human c-fos, by standard ABC immunocytotoxicological methods. c-fos immunostaining was evident in the amygdala, granular layer of the dentate gyrus of the hippocampus and in the superficial layers of many cortical areas. In the young animals the oldest group showed a decrease in c-fos expression in these areas relative to the young animals, whereas the middle aged animals exhibited no difference. This reduction was not seen in all brain areas and may serve as an indicator of brain aging. (This work was supported by NIH grant AG 07752 to W.E. Sonntag.)
480.4

REDUCTION OF RAT BRAIN SOMATOSTATIN mRNA EXPRESSION DURING AGING. T. Florio, C. Ventri, O. Meucci*, A. Scorziello*, and G. Schettini. Dept. of Pharmacology, I University Medical School, Univ. of Naples, via S. Leonardo 5, 80115, Naples, Italy.

In the past years, a growing bulk of studies demonstrated that the blockade of central somatostatin neurotransmission caused an impairment of learning and memory processes in the experimental animals. Moreover, clinical studies reported that in patients affected by the dementia of the Alzheimer's type, the somatostatinergic neurotransmission is quite affected. Thus, a primary role for the brain somatostatin in the modulation of cognitive functions has been suggested. In this report we studied whether an alteration in the brain somatostatinergic neurotransmission "naturally" occur during the aging process in the rat. For this purpose we analyzed the somatostatin mRNA expression in different rat brain areas (frontal cortex, parietal cortex, hippocampus, striatum) derived from 2, 6, 12, and 25 months old rats, by means of both northern and dot blot analysis, using pre-prosomatostatin cDNA (kind gift of Dr. R. Goodman) as probe. A clear reduction of somatostatin mRNA expression in both frontal and parietal cortex in the aged rats.

480.6

MODULATION OF TYROSINE HYDROXYLASE GENE EXPRESSION IN THE RAT ADRENAL GLAND BY RESEPRINE: EVIDENCE FOR UNCOUPLING OF TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL MECHANISMS DURING AGING. R. Strong, C. Hale, M. A. Moore and M. Weese-Boch. Department of Pharmacology and Toxicology, St. Louis University School of Medicine, St. Louis, MO 63125.

Plasma catecholamine levels are increased during aging. Because tyrosine hydroxylase (TH) is the rate limiting step in catecholamine synthesis, we are studying alterations in the regulation of TH gene expression during aging. Tyrosine hydroxylase, TH messenger RNA (TH mRNA) and TH protein were measured simultaneously in adrenal glands of Fischer 344 rats aged 2, 6, 12, and 25 months. Between 2 and 23 months TH activity rose 2 fold as compared to the youngest group. TH mRNA content of the adrenal gland rose 3 fold between 2 and 23 months. A 3 fold increase in adrenal DA content, the first catecholamine product of TH, provides evidence that the increases in TH gene expression are functionally significant.

To determine if mechanisms that regulate gene expression are altered by aging, the effects of reserpine on induction of TH mRNA and TH activity were compared in another group of rats aged 2, 12, and 27 months. Consistent with the results of the first experiment, there were age-related increases in both TH activity and TH mRNA in the age-matched control groups. TH activity rose 2 fold and TH mRNA rose more than 6 fold between 2 and 27 months. The distance in the relative magnitudes of increases in TH mRNA and TH protein suggest an uncoupling of regulation of TH mRNA and TH protein levels. Moreover, there were significant age-related differences with respect to modulation of TH gene expression by reserpine treatment. TH activity was induced by reserpine in the youngest group, but not in the two older age-groups. In contrast, reserpine caused significant induction of TH mRNA in all age-groups. These results provide evidence that aging is accompanied by alterations in TH gene expression at transcriptional and post-transcriptional levels.

480.8

VESICLES PRIMARY AXONAL TRANSPORT RATES FOR RAT SCIATIC NERVE AXONS AS A FUNCTION OF ANIMAL AGE. N.A. Kintner* and J.A. Yancopoulos. Department of Biological Sciences, University of Maryland, Baltimore County Campus, Catonsville, MD 21228.

We report the results of a pilot study of vesicle transport in aging rats. Longitudinally moving vesicles in dissected, myelinated axons from rat (Fisher 344 strain) sciatic nerves were observed by high resolution, video-enhanced contrast microscopy at constant magnification of 500 X. The rates of individual vesicles were determined by measuring the transit time through a 2.5 Xum long speed trap which was electronically superimposed on the video image of the moving vesicles. Transport rates of individual vesicles were measured for both a young (ca. 8 wks) and an old (ca. 2 y.) age group. Under these conditions, average transport rates of both anterograde moving vesicles and retrograde moving vesicles were significantly lower in the old age group.

(We thank Don Ingram and David Cissold for their contributions. This pilot study was funded by the UMGBSB SBIR program.)
480.10
BLOOD-NERVE BARRIER PERMEABILITY AND NERVE VASCULAR SPACE IN THE PERIPHERAL NERVE OF RATS OF DIFFERENT AGES. J. Kristinna, K. V. Hadzhi, A. Babko and C. Tannert. Lab. of Neurosciences, IMA, SHU, Rotherham, UK. ZTP797. The permeability-surface area products of (14C)-sucrose at the blood-nerve barrier, PA(BBB), of the sciatic nerve, and at the blood-brain barrier, PA(BBR), were determined in Fischer-344 rats at 1, 11 and 30 mo of age, using an in vivo 14C, halo injection technique and a two-time point graphical method. Nerve vascular space in the tibial nerve of these rats was also determined using quantitative morphometry. There was no significant difference of mean 97% PA(BBB) in any age group (PA(BBB)) at 3 m, +1.05FSY, ±1 41.2±13.7 m, or 11 11.2±10.7 m, or 30 25±8.2 m. 480.11
UNBIASED STEREOLOGICAL ESTIMATION OF THE TOTAL NUMBER OF NEURONS IN THE AGING Rhesus HIPPOCAMPUS
M. J. West and B.I.G. Grundseid
Stereoanalytic Research Lab., Univ. of Aarhus, Denmark
The total numbers of neurons in the dentate granule cell layer, the bilis, regio-inferior, regio superior and subiculum were estimated using unbiased stereological techniques and systematic sampling in 16 patients ranging from 15 to 85 years of age. Total neuron number was calculated as the sum of the numerical density, \( N_v \), and the reference volume, \( V_{ref} \), of the layer containing perikarya. \( N_v \) was estimated with optical dissector, \( V_{dis} \) by point counting. Sampling in both cases was carried out systematically, i.e., with a random start and a predetermined periodicity. The sampling scheme was designed so that the majority contribution to the variance of the group was the true biological difference among individuals. Preliminary data indicates that there is a significant correlation between age and neuron number in regio superior. This is an inverse relationship indicating a loss of 50% of the neurons in regio superior, but not in other hippocampal subdivisions, during adult life.
480.12
AGE-RELATED REGRESSIVE CHANGES IN MOTONEURON NUMBER AND MORPHOLOGY IN AN ANDROGEN-SENSITIVE RAT SPINAL NUCLEUS. E.M. Kurz and D.B. Sandage. Program in Neural Science, Dept. of Psychology, Indiana University, Bloomington, IN 47405.
Moteuronctes in the sexually dimorphic spinal nucleus of the bulbocavernous (SNB) in rats are sensitive to androgens during development and at adulthood. Developmentally, androgens regulate motoneuron number, somatic and dendritic morphology. In adults, reductions in androgens after castration produce significant declines in SNB motoneurons and dendritic length, and these can be restored by androgen treatment. Androgen titers decline during normal aging in male rats, and androgen-sensitive SNB motoneurons also undergo age-related changes in morphology. We examined whether this age-related, naturally occurring decline in androgens altered SNB motoneuron number, soma area, and dendritic length. SNB motoneuron number and morphology were assessed in aged (22 months old) and young adult (70 days old) rats using retrograde labeling following unilateral muscle injection with cholera toxin HRP and counterstaining with thionin. Aged and young adult males had significantly fewer SNB motoneurons than young adults (144 vs. 172); counts of HRP-labeled SNB motoneurons were also lower in aged males (45 vs. 51). HRP-labeled SNB motoneurons in aged males were significantly smaller than those of young adults in both soma area (760 vs. 843 sq µm) and dendritic length (1891 vs. 1414 µm). Weights of the androgen-sensitive SNB target muscles (bulbocavernous/elevator ani) were also significantly lower in aged males (0.92 vs. 1.23 g). These data suggest that age-related regressive changes in SNB motoneuron number, soma area, and dendritic length, and target muscle weight may result from declining androgen levels. (Supported by NIH NS24877)
480.13
SEX- AND AGE-RELATED DIFFERENCES IN MIDSAGITTAL AREAS OF THE CEREBRAL CORTEX AND CORPUS CALLOSUM: AN MRI INVESTIGATION. K. Raz, J. Sperger, and J. Porjesz. Dept. Psychology, Memphis State University, Memphis, TN 38152. We examined sex- and age-related differences in the areas of the cerebral cortex (Cx), corpus callosum (CC), and subdivisions of the CC. Mid-sagittal magnetic resonance (MR) images were obtained from 51 normal volunteers and neurologically controls (age 17 - 87). Males had larger Cx, CC, and splenial areas than females (t(49) = 6.63, p < .001, t(49) = 2.07, p < .05, and t(49) = 2.74, p < .01, respectively). However, the correlations between the areas of CC and Cx and the Cx were also significant (r = .28, p < .05; r = .33, p < .02, respectively). The Cx area is an estimate of the brain size, and as such is influenced by gender difference in body size. After controlling for this index of brain size, we found no significant effects of gender on CC or splenial areas. The Cx area, associated with age (r = .32, p < .02), was not age-related differences in CC or splenial areas. Physiological parameters measured by BRI, 550-552-26-26 and MSU Center for Applied Psychological Research.
480.14
MAGNETIC RESONANCE IMAGES OF THE CEREBRAL INFARCTION OF AN AGED RHESUS MACAQUE. H. Urap, W.D. Houser, and J.L. Holden. Wisconsin Regional Primate Research Center and Medical Physics, University of Wisconsin, Madison, Wisconsin 53715-1299.
Magnetic resonance imaging of a 30 year old, male rhesus macaque suffering from ataxia of the bilateral fore and hind limbs for approximately one year was performed at the 2nd to 3rd age of the animal was euthanized due to severe locomotive failure. The formalin-fixed whole brain was examined by MRI, with a slice thickness of 5 mm or more, resulting in the thirteenth images. The images of horizontal, coronal, and sagittal sections were obtained using TR (repetition rate) 2000 msec and echo time (echo time) 20 msec for first echo or 110 msec for second echo images. In the 2nd echo (T2 dependent) images, irregular opaque shadows were found in the left inferior parietal lobe along the lateral sulcus, right parietal lobes and bilateral cerebellar cortex. However, the images of spin density in pre- and post-mortem brain confirmed that the opaque shadows showing in T2 images were caused by CSF accumulation in widened meningeal spaces associated with a defect of the cortex (old infarction). Pathology of the brain was also examined in the commonly used lesions in the above regions. Different relaxation times [T1 - water (400-450 msec); brain cortex (250-300 msec)] could be determined by contrast using T1 and T2 images. A combination of different T2 and T1 times was necessary to determine the real nature of pathological lesions.
400.15

Aging of the Human Brain: Assessment with 1.5 Tesla MRI

P. Murai Dorsawany, Gary Fite, Mustafa Humeini, Great Britain Medical College, Sapporo, Japan.

Brain MR images of 39 normal volunteers, aged 26 to 79 yrs were used to assess neuroanatomical and biophysical changes in the aging brain. Axial MR images revealed a significant increase in the frequency of subcortical hyperintensities in older subjects. Older subjects also demonstrated significantly smaller caudate and putamen volumes compared to younger subjects. Sagittal images revealed significant age-related dimensional changes in the corpus callosum, pituitary gland, midbrain, septum, and diencephalon. Cross-sectional analyses showed a significant increase in T1 relaxation times in the hippocampus, corpus callosum, frontal and temporal white matter in the older subjects when compared with younger subjects.

These results represent the first in vivo study to simultaneously document anatomical and biophysical tissue changes in specific regions in the human brain in normal aging. Neurocognitive studies are underway in these subjects to evaluate the potential functional correlates of these findings and to establish structure-function relationships in the aging human brain.

400.16


Dietary restriction is one of the few established methods for prolonging life span in mammals, and development of several biomarkers of aging has been found to be slowed by prolonged restriction. However, much less is known about the effects of restriction on markers of brain aging. In the present studies, therefore, we have investigated the effects of long-term dietary restriction (60% of ad lib diet beginning at 16 weeks of age) on quantitative morphometric measures of aging in the hippocampus and the retina of Fischer 344 male rats. The animals were maintained by the NIA Biomarkers program at the Nat. Center for Res. Each experimental group ranged in size from 8 to 16 rats.

A gradual thinning of the retinal outer nuclear layer of photoreceptor nuclei occurs with aging in the control ad lib groups. However, the restricted diet did not influence retinal aging in 18, 21, or 27-mo-old rats, as judged by photoreceptor cell death, outer nuclear layer thickness, and pattern of cell loss. Hippocampal pyramidal cell density and astrocytic inclusions correlate doly with aging in F344 rats. In two studies, no differences in hippocampal neuronal density were found between ad lib (AL) and dietary restricted (DR) animals in the older groups (26-27 mo-old). However, in the 18-md-old and 21-md-old animals, a small (about 10%), but significant difference was found, with DR animals exhibiting higher neuronal density. In 26-mo-old rats, no significant effects of dietary condition were found on quantitative EM measurements of glial inclusions, neuronal lipofuscin, rough endoplasmic reticulum or Golgi apparatus.

These results are in contrast to studies of restriction on non-neuronal markers of aging, including basic longevity, and suggest that a different aging mechanism may modulate at least some aspects of brain aging. (Supported by AG07767)

400.17


The effect of 20 months of voluntary ethanol consumption on aging was studied in several generations of high drinking AA (Alco alcohol) rats. After the initial test of individual voluntary ethanol intake at the age of three months the rats were maintained in groups cages. One half was given free access to food, tap water, and 10% (v/v) ethanol solution, while the other half had only food and water available. The survival curves of both ethanol exposed and non-exposed groups were also pooled together or compared within the generation. No changes were found in the population longevity up to the age of 24 months when the rats were used for experiments. The ethanol exposed group consumed slightly more ethanol than the non-exposed group when measured at the age of 24 months. They did not show any difference in ethanol sensitivity when tested to produce motor impairment on the walking plane, for the duration of ethanol-induced (3.5 g/kg) loss of righting reflex, or for ethanol-induced (3.5 g/kg) hypothermia. The groups did not differ in the ethanol-induced, elimination of the concentrations of brain monoamine neurotransmitters and their major metabolites did not reveal any differences between the ethanol exposed and non-exposed old rats, while the levels tended to be higher in 3-4 month old rats. Dentricpine spine counts after Golgi-stain were not different either. The ethanol-feeding regimen used thus did not produce any measurable negative interactions of ethanol on aging.

400.18

AGE-DEPENDENT CHANGES IN VISUAL INTENSITY DIFFERENCE THRESHOLDS IN PIGEONS. P. Hussey* and W. Hodos, University of Maryland, College Park, MD 20742.

Recent studies have reported age-related deficits in visual acuity and retinal morphological in pigeons. The present report here was designed to determine the effects of age on intensity difference thresholds (IDT) in pigeons. 76 pigeons, aged 1-17 years, were trained to discriminate two stimuli that differed in luminance by 0.6 log unit. When this discrimination was established, the subjects were presented with two series of progressively smaller luminance differences. IDT was calculated from psychometric functions by determining the luminance difference that corresponded to 75% correct. The coefficient of correlation between age and IDT was +0.26, which indicates a small, systematic relationship between IDT and age; i.e., age accounts for approximately 5% of the variance in IDT. This is in contrast to visual acuity in pigeons in which age accounts for 82% of the variance. The results reported here, which are in agreement with human literature, indicate that age-related deficits in visual acuity and other visual tasks in pigeons are not due to a general deterioration of the visual system or to some non-specific global performance deficit.

400.19


Eye movement, to move the eyes in order to maintain a clear view of the central region of the retina, tells us valuable information about a human’s brain function including visual cognition. In this experiment, we investigate the development time course of eye movement on copying a figure by using a new technique, Vision Analyzer (TKK 938), experienced in 38 subjects (6-81 year old).

In 6-7 years, eye velocity on copying was diffuse to a wide range and peak, as developing the most used eye velocity was appeared and higher. The mean eye velocity was 76.8 deg/sec in 6 years, 32.6 deg/sec in 25 years and 23.2 in 81 year old. On the other hand, eye fixation was going up as developing, but it made a peak at 20-30 years old. And fixating points localized more on the model figure in young below 18 years old and on the copied figures in older than 18 years. The development time course of eye movement on copying a figure is obtained for the first time objectively, these results indicate lower eye movement in young with visual cognition and handwriting due to visual information, is to increase until 20-30 years and then hold on.

400.20


Performance of aged rats in a number of behavioral tests is disturbed when compared to young rats. It has been hypothesized that altered brain functions contribute to the reduced performance. We investigated whether the aging process affects the processing of sensory information. Young (1-4 months) and aged (33 months) rats were anaesthetized and the rat SI vibrissa cortex was exposed for single unit recording. Single whiskers were stimulated by using hand-held probes. The neuronal responses to the sensory stimulation were assigned to either of four classes according to their response vigour. It was found that in the aged rats less cells responded to the applied stimuli. Of those responding to the stimuli the neurons of the aged animals responded less vigourously, leading to a shift in the response quality distribution towards lower response qualities. Taken together, the results suggest i) that changes in cerebral function that may contribute to altered behavioral performance can be detected at the level of sensory processing and ii) that the vibrissa cortex of the rat can be a suitable model to study age-related changes in cortical function.
481.1 INCREASED PLASTICITY OF AGING MOUSE PECTINUS NEUROMUSCULAR JUNCTIONS OBSERVED IN VITRO N. Robbins, J. Hill* and R. Hill*, Dept. Neurosciences, Case Western Res. Sch. of Medicine, Cleveland, OH 44106.

In order to delineate dynamic cellular processes underlying age changes at the neuromuscular junction (NMJ), living identified NMJ's were observed at intervals of 1 week or 1 month in adult (6 mo.) and old (27-29 mo.) CB1-mice. Fluorescent stains for pre- and post-synaptic elements were employed to examine separate neuromuscular junctions, nerve terminal (NT) constuctions, and a greater area of NT outgrowth per NMJ than did adult NMJ's. Over 1 week (but not 1 mo.), more focal constuctions appeared and more pre-existing NT constuctions expanded in old NMJ. Over 1 month, NT outgrowth/retraction was greater in old NMJ, with 50% of old NMJ's (vs. 18% adult) showing 3 or more types of change, markedly so. In sum, increased focal NT withdrawal may eventually produce the constuctions and multiple regions characteristic of old NMJ's, whereas enhanced in- and outgrowth of NT may account for increased abandoned post-synaptic folds. These dynamic events could result from increased adhesion of NT to matrix and possibly increased perijunctional adhesion. Supported by NIH AG 06641.


Seasonal changes in synaptic physiology and morphology were studied in the crayfish fast clasper excitor (FCE), a phase neuron innervating the claw closer muscle. FCE EPSP's recorded from closer muscle fibers in summer animals showed a significantly lower initial EPSP amplitude (8.7 ± 1.5 mV) compared to winter animals (10.7 ± 1.7 mV, p < .02), and a significant increase in fatigue-resistance. During 30 min. of 5 Hz stimulation, the IPSP amplitude in winter animals decreased only 14%, while EPSP amplitude in winter animals decreased 92% (p < .02). In some animals, HRP was intracellularly injected into the FCE to examine terminal morphology, and it was found that the motor terminals in summer animals possess significantly more synaptic varicosities (.92 ± .10 varicosities/10 µm terminal) than those in winter animals (.40 ± .05 varicosities/10 µm terminal, p < .001).

The physiology and morphology of the FCE terminals in summer animals is similar to that seen after in vivo stimulation of the FCE in winter animals. It is proposed that high activity levels in summer animals are responsible for the seasonal change in the synaptic terminals.

481.5 DIFFERENTIAL ALTERATIONS IN VISCERAL SENSORY NEUROTRANSMITTERS OF THE NODOSE (NC) AND PETROSAL GANGLIA (PG) IN RESPONSE TO ANATOMY. C.J. Helke and A. Rabchevskiy*, Dept. of Physiology, Uniformed Services Univ., Bethesda, MD 20814.

Acute peripheral anatomy of the visceral sensory neurons of the vagus and glossopharyngeal nerves removes peripheral depolarizing and chemically influenced responses to their sensory ganglia. Whereas transmitter expression is altered by anatomy of somatic sensory neurons, anatomy-induced transmitter changes in visceral sensory neurons are largely unexplored. To study this, rats were sacrificed 1, 3, 7 or 14 days after transsection of either the cervical vagus or the glossopharyngeal nerve. The gene related peptide (G/PG) immonoreactive (ir), tyrosine hydroxylase (TH)-ir, and vaso-active intestinal peptide (VIP)-ir neurons in the respective ganglia (NC & PG) were analyzed in anoxia vs. control ganglia. In the NC, anatomy of the cervical vagus resulted in a rapid (by 1 d) reduction in the number of TH-ir cells. The expression of calcitonin gene-related peptide (CGRP) ir was dramatically increased in number by 3 days. In the PG, anatomy of the glossopharyngeal nerve similarly reduced the number of CGRP-ir cells while the local effect on CGRP-ir cells and did not increase the number VIP-ir neurons. Thus, peripheral anatomy of visceral sensory neurons differentially changed the expression of neurotransmitters in the bodies. These data have implications for immunocytochemical retrograde tracing studies with tracer application to cut axons and for studies of cultured visceral ganglia. (N02991)

481.4 EFFECT OF VARYING CALCIUM CONCENTRATIONS ON JUNCTION POTENTIALS RECORDED FROM NORMAL AND REDEEMED SYNAPSES IN THE CRAYFISH, A. ORYX*, and S.J. Veless Dep't. of Biological Sciences, Dartmouth College, Hanover, N. H. 03755.

The synaptic connections of the neurons innervating the superficial flexor muscles of the crayfish Procambarus clarkii became repressed (no junction potentials were detected under normal physiological conditions) when the nerve is cut at the center of the muscle field and the lateral fibers are removed (Ito, H., Natl. Res. Counc. Japan, 12; 1575, 1956). We suggested that calcium ions could be involved in this repression. In the present work we study the effects of changes in calcium concentrations on the junction potentials (j.p.'s) recorded from normal and repressed animals. The same type of surgery was performed to obtain a pool of repressed animals, which were have been reduced to three weeks after the operation (the peak time for repression). Analysis consisted of recording from adjacent muscle fibers with a microelectrode the j.p. generated by the spontaneous firing of neuron 3. The microelectrode was left in the fiber while changing Hinger solutions which varied in calcium concentration from 30 to 2000. Normal animals showed a 60-90% increase in j.p.'s sine with increasing calcium concentrations while repressed animals showed only a 5-20% increase. Since repressed synapses do not respond to changes in calcium concentrations as normal synapses do, this suggests that calcium is indeed involved in the mechanism underlying repression in this system.

481.6 ULTRASTRUCTURE OF "SPROUTED" SPHENICUS AFFERENTS IN THE RAT LUMBAR DORSAL HORN AFTER SCATIC DEAFFERENTATION INDUCED BY INJECTION OF THE SCATIC NERVE WITH PRUNACEA. A. El-Behy, S. E. Kapadia, and C. C. Lauterborn, Section of Neurological Surgery, Yale Univ. Sch. of Med., New Haven, CT. 06510.

We have previously demonstrated expansion of the terminal field of the saphenic nerve 4 months following deafferentation by injection of the sciatic nerve with prunaceae (Prunacea)(JON, 89). In these experiments, the sciatic nerve was injected with 20mg Prunacea; the opposite side served as a control. Four months following the injection, the saphenous nerves on both sides were injected with 0.5 mg WGA-HRP and 0.05 mg DCT-HRP, and the animal sacrificed after 48 hours. Sections demonstrating maximal expansion were prepared for EM, using the AHH (ammonium heptamolybdate) method for the peroxidase reaction. Low magnification EM montages were prepared, covering the labelled territory on the proune and the control sides. All labeled terminals were located on the montage and photographed at 10K. In each animal, 70-100 terminals on each side were classified by location and by type (number of synaptic contacts recorded) and the number of synaptic contacts made by each terminal counted. There was an increase in terminal number in all laminae on the proune side, particularly laminae III and IV. Quantitative analysis of lamina II revealed that there was an increase in the ratio of glomeruliform/simple terminals on the proune versus control side while the number of saphenic type prue on glomeruli was similar on both sides. (NIH grant NS 10174).

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
481.9 NEURAL PROPERTIES OF IDENTIFIED CORTICOSPINAL CELLS IN VIVO FOLLOWING ISCHEMIA AND NEUROTRANSMISSION block by intrathecal 6-OHDA. L. L. Lin*, M. G. Leedy and M. S. Beattie. Dept. of Anatomy and Neurobiology, University of South Carolina, Columbia, SC 29208.

We investigated the effects of ischemia and neurotransmission block by intrathecal 6-OHDA on identified corticospinal cells. The effects of ischemia and 6-OHDA were studied in the same animal. The results showed that ischemia abolished the function of the corticospinal cells and 6-OHDA block the neurotransmission. The combination of ischemia and 6-OHDA showed a synergistic effect on the function of the corticospinal cells.

481.11 WILL PRIOR SPINAL INJURY ENHANCE RECOVERY OF HINDLIMB MOTOR FUNCTION FOLLOWING SPINAL TRANSSECTION IN ADULT CATS? C. T. Lahr, H. Skene, A. P. Wanton, E. J. Gilbert, Neurosciences Research Institute, Stanford University, Stanford, CA 94305.

The present study was designed to determine if recovery from a previous spinal cord hemisection will enhance recovery of hindlimb locomotion in adult cats after a subsequent spinal transaction. Previous studies have shown a reduction of some characteristics of the response of the spinal cord to a second transaction. The present study was designed to determine if prior spinal injury enhances recovery of hindlimb motor function following a subsequent spinal transaction. The results showed that prior spinal injury did not enhance recovery of hindlimb motor function following a subsequent spinal transaction.
481.13

**EXPRESSION OF CHOLINE ACETYLTRANSFERASE (ChAT) AND NERVE GROWTH FACTOR RECEPTOR (NGFR) WITHIN HYPOTHALAMIC MOTONEURONS FOLLOWING NEUROGENIC PLASTICITY**


In the present study we employed light microscopic immunocytochemistry in order to investigate the temporal sequence of ChAT and NGFR within hypothalamic motoneurons following unilateral transection or crushing of the XII nerve. In control rats (i.e., sham operated) virtually all the motoneurons of the XII nucleus were brightly labeled for ChAT and devoid of NGFR immunoreactivity. Three days following nerve transection the intensity and the number of ChAT-positive neurites were markedly reduced on the transected side compared to the non-lesioned side. This decrease in labeling continued until nine days when virtually no ChAT-labeled cells were present on the lesioned side. In contrast, no loss of hypothalamic neurites was found using NGF antibodies. Importantly, the persistence of these motoneurons suggests that the absence of ChAT is not an absolute indicator of cell death. This absence of ChAT immunolabeling persisted for several days, yet by 30 days many motoneurons begin to re-express the enzyme. Transient (NGFR). As early as one day following the lesion motoneurons have re-expressed NGFR immunoreactivity. This response was very robust three days following the lesion and continued throughout all time points thus far examined. Importantly, NGFR immunoreactivity could be seen at times after motoneurons began to re-express ChAT. Crush (ChAT). Crushing of the hypoglossal nerve resulted in a transient reduction in the overall intensity of ChAT immunoreactivity yet produced little if any loss in the number of ChAT neurons. The decrease in labeling was most apparent 5 to 12 days following the lesion. Crush (NGFR). Nerve crush also resulted in the transient expression of NGFR immunoreactivity which was most robust 3 to 9 days following the lesion. At present the mechanism underlying the lesion induced expression of NGFR is under study.

481.14

**IMMUNOHISTOCHEMISTRY OF HYPOGLOSSAL AFFERENTS AND EFFECTS OF AXOTOMY**


Experimental Neurology, DVAMC, Nashville, TN 37212 and Vanderbilt University.

In the present study, we sought to determine which neurotransmitters can be demonstrated in the nucleus using immunohistochemical techniques and the effects of hypoglossal nerve transaction on those inputs. Adult Sprague-Dawley rats of both sexes were anesthetized and underwent unilateral hypoglossal nerve transaction with suture ligation of the proximal stump. After 3-6 d, rats were perfused with aldehyde fixative and frozen sections of the medullas processed for indirect peroxidase immunohistochemistry. Neukenpehlin and glutamate gave moderate terminal labelling in the hypoglossal nucleus. Substance P, NPY, Met-enkephalin, CRF, FMN and beta-endorphin gave light terminal labelling. DBH, VIP, CCK, neurotensin and somatostatin did not stain the nucleus. Glutamate staining was decreased in the hypoglossal nucleus ipsilateral to chronic axotomy. These results suggest several transmitter systems for studies of the reaction to axotomy or loss of motor neurons. (Supported by DVAMC.)

481.15

**C-FOS AND SILVER DEGENERATION REACTIONS IN ADULT RAT BRAIN STEM FOLLOWING ACUTE AND CHRONIC DENTAL INFLAMMATION.**


Neurobiol. & Anesth., NIDR, NIH, Bethesda, MD 20892.

We have used immunocytochemistry to analyze the expression of c-Fos and Fos related antigens in the brain stem of the rat adult at 5-6 hr after pulp exposure injury to the right maxillary first and second molars. The acute group was compared to others with the same injury that survived 5-6 days or 15 days. Postoperative behavior and weight gain were normal after this surgery. After fixation alternate transverse sections were processed for Fink-Heimer silver stain or immunocytochemistry. Three different response patterns were found. Acute Fos/Fra response included many ipsilateral caudal neurons, fewer bilateral caudal neurons, plus many bilateral stained neurons in the periventricular lamina X, medial and lateral solitary nuclei and area postrema. Chronic Fos/Fra response only occurred in caudal neurons and a cluster of neurons in the rostral solitary nucleus at the level of the intercolliculus, with a predominantly ipsilateral response. Silver Degeneration was not prominent in the Fos reactive sites but was located more rostrally, especially in intercollicular/oral areas. These distinctive responses to dental inflammation in the rat offer a useful model for analysis of central plasticity in response to peripheral inflammation. Supported by NIH grants DE01519 and DE04942.

481.16

**CAPILLARY GROWTH IN CEREBELLAR CORTEX OF ADULT RATS AFTER EXTENSIVE PHYSICAL EXERCISE.**


Previous work has demonstrated new capillary formation in visual cortex of rats in association with synaptogenesis and tissue volume expansion. (e.g., Black, et al, Neurosci Lett. 85,351, 1987; Black, et al, Neurobiol Aging. 10:353, 1989). Angiogenesis might also occur when neurolip metabolism is chronically elevated, such as repetitive activation of neurons and synapses. We examined microvasculature in the paramedian lobule (PML) of the cerebellum of 38 adult female rats put in 4 experimental groups for 30 days. Acroinetic Condition (AC) rats were given extensive visuomotor training on difficult tasks, e.g. traversing rope ladders and thin dowels. This group tested effects of learning on synaptic connectivity. Forced exercise (FX) rats ran on a treadmill, and those in the Voluntary exercise condition (VX) had free access to a running wheel. Inactive Condition (IC) rats did not have access to either learning or exercise. Sections of PML were drawn using a camera lucida, and the area density of blood vessels in the molecular layer was determined. Previous work demonstrated substantial synaptogenesis and volume expansion in the AC rats and none in the other groups. The significant increase in vessel density in the FX and VX groups without changes in neurolip volume indicates infiltration of this tissue by new blood vessels. The PML is activated by limb movements, and presumably the increased metabolic demand in the exercise groups elicited compensatory angiogenesis. Supported by NIMH 45320, MH18822, MH16142, HD07353, and Stroke Council of the AHA.
482.1

BIOCHEMICAL COMPARISON OF ASTROCYTE MATURATION IN VITRO AND IN VITRO.

George M. Smith1, James Jacobberger2, and Robert H. Miller2. 1Dept. of Neurosurgery, Baylor College of Medicine, 6550 Fannin, Houston, Texas 77030; 2Dept. of Neuroscience, Case Western Reserve Univ., Cleveland, Ohio 44106.

The ability of astrocytes to support axon outgrowth diminishes during maturation of optic nerve. The reduction also occurs as cultured astrocytes aged in vivo. Several molecules, including NCAM and laminin have been demonstrated by antibody inhibition studies to be involved in astrocyte mediated axon outgrowth. In this study, the expression of HNK-1, NCAM, laminin, and GAPF was compared during in vivo and in vitro astrocyte maturation by indirect immunofluorescence, and analyzed by flow cytometry. In parallel experiments, maturing rat brains were similarly labeled and examined. The results indicated that at birth the majority of cortical astrocytes expressed high levels of the three cell surface molecules. While number of astrocytes expressing HNK-1 and NCAM diminished, both in vivo and in vitro, during astrocyte maturation, there was no significant decrease in the number of astrocytes expressing laminin. To distinguish between differentiation of a single astrocyte population or differential replication of multiple astrocyte populations, in vitro samples were labeled for NCAM or HNK-1, counter stained for DNA content, and analyzed by flow cytometry. Cell cycle phase fraction analysis indicated no significant difference in replication rate between those astrocytes that did and did not express HNK-1 and NCAM. Therefore, the reduction in HNK-1 and NCAM expression continues with reduction in axon outgrowth promoting properties of astrocytes. Furthermore, using the above markers, astrocyte maturation appears temporally and biochemically indistinguishable in vivo and in vitro.

482.2

FGF and PDGF Differentially Regulate the Developmental Course of Oligodendrocyte Progenitors Placed in Culture. A.L. Gard and S.E. Pfeiffer. Department of Microbiology, University of Connecticut School of Medicine, Farmington, CT 06032.

Three consecutive stages of the oligodendrocyte (OL) lineage have been distinguished: mononuclear, in which the cell body is prominent; bipolar, when the cell body has become indistinct; and with appearance of cell surface antigens, A2B5, O4 and galacocerebroside (GalC). Recently we demonstrated that OL progenitors bearing the A2B5*O4*GalC- phenotype, termed "prolondrocytes," express a distinct phenotype when placed in culture. We have compared these cells to differentiated cells in a chemically defined medium to sequentially express GalC and the major myelin proteins (Gard and Pfeiffer, Development 106: 119, 1989).

To examine whether this stage is targeted by mitogens, in OL development, prolondrocytes purified directly from postnatal telencephalons were cultured under defined conditions with either basic fibroblast growth factor (FGF) or plant-derived growth factor (PDGF) added at the time of seeding. At physiologically relevant concentrations, FGF acted as a potent mitogen for multipolar prolondrocytes (ED50 = 1 ng/ml), producing a maximum 5-fold increase in bromodeoxyuridine labeling index (20% in 6 hrs) compared to control cultures (4%) in plateau levels (2-5 ng/mg). With alcohol the time of differentiation into GalC-OL (30% by 1 DIC, 80% at 2 DIC). In contrast, PDGF caused >90% of the prolondrocytes to transiently revert to 1 DIC cells resembling their precursors (ED50 = 3 ng/ml), characterized by an A2B5*O4* phenotype and bipolar morphology. Reverted cells proliferated, ex-expressed O4 on their surface after 1-2 days as bipolar cells, and finally reverted and differentiated on a delayed schedule. The reversion was verified by single cell analysis, and also appeared in cultures of dissociated 7-day optic nerve cells, which otherwise utilize FGF as their mitogenic signal. Supported by NIH Grant NS10861.

482.3

MHC ANTIGEN EXPRESSION IN THE RAT RETINA FOLLOWING INTRACRANIAL OPTIC NERVE SECTION. K. Raul D. Radel and E.D. Lund. Department of Neurobiology Anatomy and Cell Science, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Previous studies (Streed, WJ et al., Exp. Eye Res. 1989) have shown that axotomy of motoneurons causes expression of both class I and class II MHC antigens on microglia around cell bodies. We have examined here, MHC antigen expression in the adult rat retina after intracranial optic nerve section, using the opposite (unlesioned) eye as a control. In this system, regeneration does not normally follow axotomy and the microglia are present in the normal retina. The animals were fixed at regular intervals following optic nerve section and cryostat sections were stained with antibodies against microglia and MHC antigens. Within 3 days of axotomy, microglia within the inner plexiform layer express class I MHC antigens. By 11 days the majority of expressing cells are closely associated with the optic fiber layer. Although cells in the choroid in the normal and experimental retina express class II antigens, no staining of class II-positive cells was seen in any of the retinal layers.

This suggests that under conditions of retrograde degeneration, like those in orthograde degeneration (Smetanka et al., Brain Res. in press), expression of MHC class I antigens can be present without class II expression. It also indicates that expression patterns associated with retrograde degeneration vary depending on the system studied.

Supported by The Winters Foundation.

482.4

ASTROCYTES RELEASE A NITROSYL COMPOUND WITH VASORELAXANT PROPERTIES. S. Murphy and G. Wels*. Dept. of Pharmacology, College of Medicine, Univ. of Iowa, Iowa City, IA 52242.

Astrocytes release a variety of vasoactive eicosanoids. In addition, these cells release a non-prostanoid, labile vasodilator in response to bradykinin (Murphy et al., J. Neurochem. 54, 1990) which we have termed astrocyte-derived relaxing factor (ADRF). This factor is nitric oxide (NO) or a nitrosyl compound derived from arginine. To investigate further the regulation of ADRF release, primary astrocyte cultures were exposed to a variety of agents which interact with surface receptors or with intracellular effectors, and NO release determined by a chemiluminescence technique.

Quinacrine and nor-prednisolone, but not a range of other receptor agonists, were effective in evoking NO release from astrocytes. The noradrenergic effect was not inhibited by 8-receptor antagonists, was not mimicked by cindione or blocked by guanethidine. Dade NO sensor was reversed by the 8-receptor agonist prazosin. AMPA did not mimic the effect of quinacrine, suggesting involvement of the metabotropic excitatory amino acid receptor. Voltage-sensitive A2B5+ O4'- OL astrocytes released NO as well as the non-phorbol tumor promoter which selectively discharges intracellular calcium, also evoked NO release. Thus, astrocytes, like endothelial cells and neurons, release a NO-containing compound which could potentially interact with soluble guanylate cyclase in adjacent cells, and so act as an intercellular signal.

482.5

ACTIVATORS OF PHOSPHOLIPASE D IN ASTROCYTES. G. Briner and S. Murphy. Dept. of Pharmacology, College of Medicine, Univ. of Iowa, Iowa City, IA 52246.

Astrocytes synthesize and release eicosanoids upon stimulation with purinergic agonists and agents such as calcium ionophore and phorbol esters. The precise mechanism by which this process occurs is unknown. We would involve phospholipase D (PLD). To determine the mechanism of PLD activation, and learn more about its potential role in eicosanoid production, primary astrocyte cultures were incubated with [3H]myristate, and the formation of phosphatidylethanolamine (PE) was measured in ethanol after stimulation with various agents for 30 min. Formulation of PE by phorbol myristate acetate (PMA; EC50=6mM) was abolished by pretreatment with phorbol ester (PE) receptor agonists which might be predicted to stimulate PLD through activation of PKC include TPA, bradykinin, glutamate, and carbacol; each of these agents failed to stimulate PE in astrocyte cultures. Thapsigargin (Tg), a novel compound which has been reported to selectively discharge intracellular calcium stores but not activate PKC, mobilized arachidonic acid and evoked thromboxane A2 production in dose dependent manner (EC50=12nM). In Tg stimulated PE formation, however, the effects of Tg on PLD were abolished when PKC was first down regulated. It is apparent that PLD is not coupled to surface receptors in astrocytes, including purinergic receptors which are known to be linked to eicosanoid production. Physiological regulators and the role of PLD in astrocytes have yet to be defined.

482.6

SV40 T ANTIGEN MEDIATED IMMORTALIZATION PRESERVES THE NEURITE OUTGROWTH PROMOTING PHENOTYPE OF CNS ASTROCYTES. P.S. Fria*, M.N. Goodman, G.M. Smith, J. Silver and J. Jacobberger, Case Western Reserve University, Dept. of Genetics and Neurosciences, Cleveland, OH 44106.

CNS maturation is accompanied by an attenuated neural repair capacity that is correlated with a change in the neurite outgrowth promoting properties of astrocytes. Similarly, the outgrowth promoting properties of astrocytes in vitro is dependent on the environment of the animal at the time of axotomy: isolation as well as length of time in culture. In general, immortalization by single dominant oncogenes correlates with inhibition of cellular differentiation. Thus immortalization of astrocytes at time intervals from the maturing brain should yield cell lines trapped in differentiated states representative of the maturing CNS. This hypothesis has been tested by transfecting rat and mouse cortical astrocyte cultures with SV40 large T antigen.

Morphologically, the resulting cloned cell lines are type 1 astrocytes. Many express GFAP and T antigen, are contact inhibited, and capable of entering a quiescent cell cycle phase. These cell lines have been tested for their ability to promote neurite outgrowth using embryonic chick retinal ganglion cells. Clonal lines derived from aged primary mouse astrocyte cultures expressed a range of abilities to promote neurite outgrowth, however, the mean value for all matrue lines tested was equivalent to primary aged cells. Two cell lines derived from primary rat day 2 cultures both supported neurite outgrowth at the same level as primary day 2 day cultures.

Thus, it has been demonstrated that SV40 T-immortalized astrocytes are phenotypically similar to the differentiating astrocyte population extant in the maturing CNS as the time of isolation and culture, that the immortal phenotype is stable, and that the cells are relatively non-tumorigenic in transformation phenotype.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
482.7 NEUROTRANSMITTER REGULATION OF GLYCOGEN METABOLISM IN CULTURED ASTROGLIA. O. Song*, D. L. Feinstein, and P. J. Maguire*. Institut de Physiologie, Faculte de Medecine, Universite de Lausanne, CH-1005, Lausanne, Switzerland.

In recent years astrocytes have been shown to possess receptors for various neurotransmitters. These observations further support the notion of neurotransmitters functioning in neuronal interactions between neurons and astrocytes. We have previously demonstrated that NA, VIP, adenosine (all via cAMP) and cholera toxin induce the synthesis of H-glucose in subconfluent cultures of astrocytes prepared from neonatal mice. Cultures were grown for 2 to 3 weeks in DMEM containing 10% FCS and 25 mM glucose. On the day of experimentation, FCS was removed and glucose levels decreased to 2.5 mM; after 4 hrs, substrates under scrutiny were added for 30 min. Endogenous glucose was measured by the hexokinase-glucose-6-phosphate dehydrogenase fluorometric procedure following amylase/tissue treatment of the samples. In agreement with our observations in slices and with early experiments in cultured astrocytes in which glucose had been pre-labeled with H-glucose, NA (100 mM) and VIP (1 uM) hydrolyzed 70% of endogenous glucose; other active agents included adrenaline, isoproterenol, dopamine, dbcAMP and the phosphor ester PDBu, all at 100 nM. In contrast to slices, K+ at 25 mM was inactive; however, in the presence of the Ca-channel agonist BAY-K 8644, K+ elicited ~40% glucose hydrolysis. A glycogenolytic response to K+ (~30%) was also observed in cultures treated with 1 mM dbcAMP for 3 days prior and up to 5 hrs before the experiment. This treatment also results in increased (4-4 fold) glycolytic levels and in a decreased response to NA, VIP and adenosine. The mechanism(s) of this “desensitization” is currently being investigated.

482.8 VIP RECEPTORS AND VIP-STIMULATED CAMP FORMATION IN CULTURED ASTROGLIA AND CEREBRAL MICROVESSELS. D.L. Feinstein, C. Rossier*, N. Yu*, J. L. Martin, P. J. Maguire. Institut de Physiologie, Faculte de Medecine, Universite de Lausanne, CH-1005, Lausanne, Switzerland.

In mouse neocortical slices, VIP interacts synergistically with norepinephrine (NA), adenosine and phorbol esters to increase cAMP levels. However, it is not known which cell type(s) are the target(s) for these interactions. We have therefore begun a series of studies in culture and acutely isolated tissue from both of these preparations a synergistic response occurs. We first characterized VIP binding to cultured astroglia. Specific binding of [3H]VIP was rapid, saturable, and reversible, and revealed the presence of 2 types of binding sites, a high affinity site with a Kd of 1 nM and Bmax of 150 fmol/mg protein, and a low affinity site with a Kd of 85 nM and Bmax of 150 fmol/mg protein. The related peptide PHI inhibited VIP binding with an IC50 of 10 nM, while acetyl at up to 1 uM had no effect on VIP binding. The presence of 0.1 nM VIP for 30 minutes elicited an increase in cAMP levels (4-8 fold over basal levels). Increases were also observed with NA (10 nM) and adenosine (7.5 nM), but not with the phorbol ester PDBu. Preliminary experiments indicate that all 3 of these molecules interact synergistically with VIP. Serotonin deprivation for 4 days caused loss of the synergism with NA and adenosine, whereas with PDBu maintained. In acutely isolated mouse cerebral microvessels, both NA and VIP elicited increases in cAMP (EC50 of 50 nM and 50 nM, respectively). However, synergism was found as the response to the combination of these drugs was the sum of the individual responses.

482.9 INITIATORS OF GLYCOPROTEIN PROCESSING GLUCOSIDASES BLOCK OLIGODENDROCYTE DIFFERENTIATION. R.R. Shah. Department of Biochemistry and Sanders-Brown Center on Aging, University of Kentucky College of Medicine, Lexington, KY 40536.

Previous studies (Shah et al, J Neurosci Res 29, 158, 1988) have shown that inhibitors of processing glucosidases reversibly inhibit oligodendrocyte (OD)-specific activities in heterogenous cultures of developing rat brain cells. The present study examines the direct effect of processing inhibitors on proliferation and differentiation of isolated OL progenitor cells. The OL progenitor cells isolated from mixed glial cultures of newborn rat brain undergo an initial period of active proliferation (peaking 2-3 day post-plating) followed by a marked induction of differentiated properties of OL, i.e. sulfogalactolipid synthesis and 3',5'-cyclic nucleotides 3'-phosphohydrolase activity. Castanospermine (Cst), a potent inhibitor of processing glucosidases inhibited the induction of these activities without affecting cell proliferation. The effect was concentration-dependent with 60-400 nM inhibition observed at 250 mM Cst. Swaissonine, an inhibitor of processing nanosidase, had no effect on OL differentiation even though, like Cst, prevented the formation of complex-type oligosaccharides. It is concluded that core glycosylation and initial processing of oligosaccharides may be critical for the functioning of specific glycoproteins essential to OL differentiation. (Supported by NNSF grant # NS19279)


We have previously demonstrated that grafts of unoriented pieces of E14 fetal spinal cord in C3 neocongenic nude (C57BL/6J) recipients ameliorate the deterioration of hindlimb performance. We now report that the effects of such grafts are not as maximal as those observed from video tapes. In a "blind" experimental design, trained animals were coded and laminectomy performed at C3. The C57F1 was bilaterally sectioned at the rostral and caudal borders of the segment and then aspirated. Seven subjects were chosen at random to receive grafts of 10 cultured E18 rat astrocytes. Lesion-only and grafted subjects were tested at 14, 30 and 45 days. Prelesioned animals had 3 hindlimb slips in 20 traverses. At 14 days, lesion-only animals had 10 slips whereas grafted animals had 40 slips. Slips remained at these levels to 45 days. Astrocyte grafts placed in the lesion pocket resulted in decreased rather than increased hindlimb performance as was previously demonstrated using unoriented whole spinal cord grafts. Support: Veterans Affairs


Gomori-positive astrocytes have been identified in the periventricular interstitial astrocyte cultures on the basis of their endogenous peroxidase activity, affinity for chlor am hematoxylin, and orange-red autofluorescence of differentiated fetal rat astrocytes in tissue cultures, astrocytes containing cytoplasmic inclusions with the above tinctorial and fluorescent properties represented less than 1% of GFAP-positive astrocytes at day 15-18. There was a marked increase in the fraction of Gomori-positive astrocytes and their granule content between 10 and 46 DIV. The results of the peroxidase-antiperoxidase for their peroxidase-induced cytoadherence of either mouse or human astrocytic cultures appeared to be non-enzyme-mediated insofar as it catalyzed dianioninbin oxidization over a wide range of pH (3-11) and could not be inhibited by tissue pre-heating or the calcium inhibitor, aminotriazolo. Metallo-porphyrins probably mediate both the pseudoperoxidase activity and autofluorescence in these cultures. Alterations of the redox environment and modifications of porphyrin/base biosynthetic enzymes may be the mechanisms responsible for this cytoadherence effect.

482.12 INTERCELLULAR PH REGULATION IN CULTURED ASTROCYTES: A MICROELECTRODE STUDY. W. Waltz and W. H. Witting*. Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, SK S7N 0W0, Canada.

Transmembrane transport processes involved in regulating intracellular pH in astrocytes were studied. Primary cultures of fetal rat astrocytes and Swiss mice were impaled with two-channel pH-sensitive microelectrodes. In bicarbonate-buffered saline pH3 was 7.05 and in HEPES-buffered saline cultures, astrocytes containing cytoplasmic inclusions with the above tinctorial and fluorescent properties represented less than 1% of GFAP-positive astrocytes at day 15-18. There was a marked increase in the fraction of Gomori-positive astrocytes and their granule content between 10 and 46 DIV. The results of the peroxidase-antiperoxidase for their peroxidase-induced cytoadherence of either mouse or human astrocytic cultures appeared to be non-enzyme-mediated insofar as it catalyzed dianioninbin oxidization over a wide range of pH (3-11) and could not be inhibited by tissue pre-heating or the calcium inhibitor, aminotriazolo. Metallo-porphyrins probably mediate both the pseudoperoxidase activity and autofluorescence in these cultures. Alterations of the redox environment and modifications of porphyrin/base biosynthetic enzymes may be the mechanisms responsible for this cytoadherence effect.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990 THURSDAY
462.14 THE VARIABLE MITOTIC RESPONSE OF ADULT RAT OLIGODENDROCYTES RS VICK and GH DeVries Dept Biochemistry, Medical College of Virginia, Richmond, VA 23298
Adult oligodendrocytes (OLGs), isolated and cultured according to Vick, et al. (Neurosci Lett. 35:524-534, 1984), were stimulated with putative mitogenic (P)X or co-cultured with dorsal root ganglion (DRG) neurites for various times up to 6 d. Rh tyramine was added for the final 48 h in the DRG co-culture experiments. Previous studies demonstrated that adult OLGs are virtually unresponsive to most soluble and particulate mitogens (Vick and DeVries, J Neurosci, Res submitted 1990). Fluorescence activated cell sorting (FACS) demonstrated that 100% of the unstimulated cultured OLGs were quiescent (G1/G0) state. Autoradiography demonstrated that 20% of the PX stimulated OLGs proliferated after 3 d; FACS analysis of the OLG cultures demonstrated that this proliferation was not synchronized. Co-culture of OLGs for 2, 3, and 4 d yielded a labeling index (LI) of 7%. By 5 d the LI increased to 25% and by 6 d 40% of the cells were labeled. The variability in the lag of the mitogenic response of the adult OLGs indicates that they are in variable states of responsiveness to mitogens. Thus the morphologically and immunologically homogeneous population of adult OLGs respond to mitogens in a heterogeneous manner. (Supported by NIH NS07288 and MS Society 5073)

462.15 DYSMYELINATION IN BOVINE E-MANNOSEIDOSIDOSIS: WHITE MATTER LESIONS IN A LYSOZOMAL STORAGE DISEASE IN SALERS CALVES KL Lovel, MZ Jones, and B. Abbott* Dept Pathology, Mich. State Univ., E Lansing, MI 48824 and Texas Veterinary Medical Diag. Lab. College Station, TX 77841
An inherited defect in lysosomal s-mannosidase activity has been previously reported in goats and humans. Affected goats are unable to rise and show neurological deficits, including restricted movements of the limbs, and have difficulty breathing. In the human cases there have been mental retardation and deafness. Pathological changes in affected goats include cytoplasmic vacuolation and myelin deficits, with consistent regional variation. In this study of bovine s-mannosidosis, the glial cell and myelin abnormalities were investigated in Salers calves recently identified as affected with e-mannoseidosis. Rabies infected tissues from three affected animals and one control animal were examined. Paraffin-embedded sections were stained with hematoxylin & eosin and with luxol fast blue-periodic acid Schiff-Holmes stains. Semi-thin Epon-embedded sections were stained with toluidine blue. In the cerebral hemispheres, there was a substantial decrease in the volume of white matter in the affected calves. Myelin deficiency was apparent throughout the brain, with marked variation in severity among regions. For example the myelin deficit was more severe in the corpus callosum, where practically no myelin sheaths were present, than in the cerebellar white matter. The spinal cord showed much greater amounts of myelin than brain regions examined. In regions of severe myelin deficiency, glialia, with an increase in astrocytic processes, were prominent. The regional pattern of white matter lesions in bovine s-mannosidosis was similar to that previously reported in canine s-mannosidosis and very different from the pathological changes reported in bovine a-mannosidosis, the most closely related lysosomal storage disease. The specific causal relationship between s-mannosidase deficiency and myelin deficits has not yet been defined. Supported by NS 20254 to KLL and NS 16868 to MZJ.

462.16 NEUROTELE OTUROSION PROMOTION BY RAT OLFACTORY BULB CULTURES AND CELL LINES. M. N. Goodman, J. Silver and J. W. Jacobberger. Dept. of Genetical and Neurosciences, Case Western Reserve University, Cleveland, OH 44106
The rat olfactory bulb (OB), an area of the brain re-innervated throughout life, contains two glial cell types within the outer lamina and glial limitans. Both cell types are intermingled, but sensory axons associate preferentially with ensheathing bulb cells. When the adult OB is lesioned, bulb cells at the lesion site form a glial scar, however, re-innervation occurs by growth of axons around the scar. To investigate the role of each cell type in adult bulb re-innervation, the neurite outgrowth promoting ability of immortalized adult OB cell lines, neonatal and adult OB glial culture (CC) astrocyte cell lines, and CC astrocyte cultures were measured and compared. Neuronal OB and CC cultures promoted outgrowth equivalently. Adult OB cultures promoted outgrowth at an intermediate level between neonatal OB cultures (the highest observed levels) and adult CC cultures (the lowest levels). Adult ensheathing cell lines promoted higher levels of outgrowth than adult ensheathing lines derived from either OB or CC, and were equivalent to or greater than OB and CC neonatal neurites. Neurite outgrowth over adult OB and CC astrocyte lines was equivalent to or lower than that over adult OB cultures.

These results suggest that ensheathing cells retain a high level of axon growth-promoting capacity relative to OB astrocytes, and that the neurite outgrowth capacity of OB astrocytes, like CC astrocytes, is reduced during maturation. Purification of ensheathing cells and astrocytes from OB cultures for neurite outgrowth studies is in progress.
482.19
MURINE MONOCLONAL ANTIBODIES SPECIFICALLY KILL A HUMAN GLOBLASTOMA CELL LINE IN VITRO. C. Matute, Y. Sánchez-C., C. Rico, R. Conde and E. Simón, 1st Department of Neurosciences, University of País Vasco, 48940-Leioa; 2nd Department of Morphology, University of Zaragoza, 50009 Zaragoza, Spain.

We have produced murine monoclonal antibodies (mAb) to a human glioblastoma cell line (T2) recently established in our laboratory. Balb/c mice were immunized using as antigens 2×106 of T2 cells grown in nude mice. mAbs binding to T2 cells but not normal human central nervous tissue were tested to test their cytotoxic effects on T2 cells in vitro. *H*-thyridine incorporation and total protein content assays showed that mAb 1A1-B7 and 1D4-G3 were able to alter the normal growth of T2 cells. However, these mAbs did not significantly affect the growth rate of other malignant human cells like rT cells or rat primary astrocytes. An hour incubation with 3 μg/ml of ammonium sulphate precipitated ascitic fluids containing mAb 1A1-B7 followed by 0.5 mg of rabbit complement applied for two hours was sufficient to kill 74% of T2 cells and 23% of the rat glioma cell lines; whereas cultured astrocytes grew as in control wells. Irrelevant mAbs, complement itself or a combination of both were not cytotoxic at these concentrations. Preliminary immunoblot experiments indicate that mAb 1A1-B7 and 1D4-G3 recognize a 200 Kd band. These mAbs bind to a T2 molecule which might show some partial homology to a molecule present in rat glioma C6. The mAbs reported here are specific for a human glioblastoma cell line and they might be valuable immunotherapeutical probes.

This work was supported by DGICYT (PM-88-100).

482.20
THE RETINAL PIGMENT EPITHELIUM (RPE) GENERATES AN INCREASE IN SUBRETINAL SPACE VOLUME IN FROG RETINA. B. Huis and C. J. Karasiewicz. Vision Research Laboratory, Department of Psychology, University of Georgia, Athens, GA 30602.

Membrane impermeable ions, combined with ion-selective electrodes, can be used to measure changes in extracellular fluid volume. We have recorded AECS in the subretinal space (SRS) in Rana pipiens during normal light- and dark-adaptation. Eyecups were superfused with the ECS marker ions tetramethylammonium (TMA) or tetracaine/lidocainum, or the amine hexadecaneamine or naphthenamine sulphonate. A light- and dark-adapted increase in concentration of these ions was measured. This increase is caused by an expansion of the intraretinal fluid space, because: (1) The amplitude and time course of cation and anion responses are similar; (2) The TMA response recovers to baseline during a long-duration stimulus; (3) The resistance across the RPE is decreased by light. Glutamate analogues (2-amino-4-phosphonobutyrate + kynurenic acid or aspartic acid) do not block the TMA decrease, which indicates mechanisms postsynaptic to photoreceptors are not involved. A TMA decrease is not observed in the isolated retina, therefore the RPE is involved in generating the TMA decrease. The TMA decrease is depressed by 2H+, a K+ channel blocker, but is not affected by blockers of active transport mechanisms.

We conclude that during light the RPE cells shrink, leading to an increase in SRS volume. Light- and dark-adapted AECS will affect extracellular concentrations of all substances.

Supported by NIH grant EY-05276.

482.21
ISOLATION OF cDNAs EXPRESSED IN THE ADULT MOUSE CHORDOID PLEXUS. E. Lee*, T. Rhyner, J. M. C. Mucci and B. Persac. Centre de Biologie Cellulaire, CNRS, 67 rue Maurice Günzburg, 94205 Ivry sur Seine cedex, France.

We have previously reported the construction of a subtracted cDNA library from a mouse cerebellum astroglial cell line (Rhyner, T. et al., J. Neurosci. Res., in press). The recombinants isolated from this library, whose sizes range from 100 to 200 bp, have been characterized by nucleotide sequencing and the pattern of expression in various mouse adult tissues and regions. Two recombinants code for smooth muscle α actin and fibronectin.

The sequences of two other recombinants do not reveal any significant homology with any cDNA described to date when compared with data bases. These recombinants hybridize to mRNAs present in the brain and in various tissues including kidney, testis and muscle, as well as in primary cultures of cerebellar astrocytes. To identify the cell types responsible for mRNA expression, in situ hybridization experiments were carried out with RNA probes. The antisense RNAs gave a clear signal in choroid plexuses of the lateral and 4th ventricles as well as in few ependymal cells. In addition, one recombinant hybridized to limited areas of kidney cortex, i.e. some glomeruli cells and the proximal tubule.

These results show that the strategy used in this study has led to the isolation of undescribed cDNAs that hybridize to mRNAs expressed in astrocytes and other cell types outside the CNS.

(Supported by CNRS, MRT, ARC and FRMF).

482.22
EVIDENCE THAT NORMAL ASTROCYTES DO NOT MIGRATE UNDER NON-INVASIVE (NON-TRANSPLANT) CONDITIONS. J.D. Hatton, J.P. Finkelson & H.S. U., 1st Div. of Neurosurgery, UC San Diego, La Jolla, CA.

Previous research has shown that grafted astrocytes have the ability to migrate throughout most of the rat central nervous system. Reactive host astrocytes have been shown to migrate into superior central ganglion autografted into the brain as target tissue. However, the ability of normal astrocytes to migrate under non-invasive conditions has not been explored. We investigate this question, host astrocytes must be labelled without invasive damage. Thus, rats were anaesthetized and placed in a stereotaxic. A Burr hole in the skull exposed the dura mater, which was pierced with a surgical microscope. The pine was gently punctured several times with the tip of a 31g needle. The area was overlaid with gelfoam containing fluorescent polystyrenes in tissue culture medium. After two hours, the gelfoam was removed and the wound was closed. After 2-4 weeks of recovery, rats were perfused with aldehydes and their brains removed and sectioned on a cryostat for fluorescence histology.

Fluorescent polystyrenes were taken up by both piloblasts and astrocytes at the pial-gial margin. Labelled astrocytes identified by GFAP staining were confined to the area of the original labelling site, and did not migrate either laterally across the pial margin or ventrally into the cortical layers. Labelled astrocytes did not appear to be either hyperplastic or hypertrophic. Although minimal damage may have occurred during labelling, these results suggest that astrocytes do not migrate in the absence of some invasive stimulus.

483.1
ROLE OF AP2 AND NEGATIVE REGULATORY CIS-ACTING ELEMENT IN GLUTAMINE SYNTHETASE EXPRESSION. R.J. Piché*, R.G. King and J.F. Mill. Lab. of Molecular Biology, NINDS, NIH, Bethesda, MD 20892.

Glutamine synthetase (GS) plays a central role in nitrogen metabolism and ammonia detoxification in the central nervous system, where its expression is confined to astrocytes. The gene for GS is cloned and the 5' flanking region isolated. A series of deletion and site-directed mutants of the promoter region were generated by excision of various mouse adult tissues and regions. Two recombinants code for smooth muscle α actin and fibronectin.

The sequences of two other recombinants do not reveal any significant homology with any cDNA described to date when compared with data bases. These recombinants hybridize to mRNAs present in the brain and in various tissues including kidney, testis and muscle, as well as in primary cultures of cerebellar astrocytes. To identify the cell types responsible for mRNA expression, in situ hybridization experiments were carried out with RNA probes. The antisense RNAs gave a clear signal in choroid plexuses of the lateral and 4th ventricles as well as in few ependymal cells. In addition, one recombinant hybridized to limited areas of kidney cortex, i.e. some glomerulus cells and the proximal tubule.

These results show that the strategy used in this study has led to the isolation of undescribed cDNAs that hybridize to mRNAs expressed in astrocytes and other cell types outside the CNS.

(Supported by CNRS, MRT, ARC and FRMF).

483.2

To determine the distribution of fos immunoreactivity induced by the dissociative anesthetic ketamine (KET), rats were injected with ketamine (50 mg/kg) and were perfused 2 hr later with 4% paraformaldehyde plus 0.2% glutaraldehyde. 30-μm thick sections were stained for fos protein. A prominent band of immunoreactive nuclei was detected in posterior but not anterior cingulate cortex. Quantitation of positively stained nuclei revealed maximal expression at 80 mg/kg. Nal attenuated but did not eliminate staining for fos. Other areas exhibiting fos immunoreactivity were: a) central n. amygdala, b) n. edinger-Westphal, c) paraventricular, supramamillary, centromedian, and dorsomedial n. of hypothalamus, d) substantia nigra, and e) layers IV and V of cortex. No staining was noted in neocortex or hippocampus. These results suggest that ketamine may alter the metabolism of neurons involved in the affective component of pain perception.
483.2

STRONG EVOLUTIONARY CONSERVATION OF NEUROPEPTIDE Y; CHARACTERIZATION OF SHARK GENE. D. Latham, G. McIndoe and T. Lundell. Department of Medical Genetics, Uppsala University, Box 580, S-751 21 Uppsala, Sweden.

Neuropeptide Y (NPY) is an abundant and widely distributed neuropeptide in the CNS and PNS of all mammals investigated. NPY potently inhibits release of neurotransmitters, notably blood pressure and food intake which are both increased by NPY. NPY is a member of a peptide family which also includes peptide YY (PYY) and pancreatic polypeptide (PP) as well as the fish peptide proenkephalin-A.

To investigate the degree of conservation of NPY at the structural level during evolution, we have recently isolated DNA clones encoding chicken and goldfish NPY. We have previously reported that chick and goldfish NPY are highly related as they display only 1 and 5 differences from the human sequence. The shark sequence displays no unique amino acid residues as compared to the other known NPY sequences. This suggests that shark NPY may be identical to NPY of the common ancestor of cartilaginous fishes, bony fishes, and mammals.

We are now in the process of characterizing lamprey cDNA clones and screening a Torpedo cDNA library. The impressive similarity of shark NPY to human NPY makes this peptide one of the most highly conserved known; 92% identity.

483.3


Distinct subtypes of the GABA_b benzodiazepine receptor have been characterized in the mammalian CNS using ligands which interact with the benzodiazepine recognition site on this protein. Recent evidence from combinations of GABA_b receptor subunits expressed in transfected mammalian cells suggest that type I benzodiazepine receptors, as characterized by high affinity for CLB 218 and methyl β-carboline-3-carboxylate are associated with the α6 subunit, while type II receptors correspond to a low affinity for the above two ligands, are found if α1 is replaced by α2 or α3 subunits in the transfected cells.

In order to delineate these further, we have exchanged the N-terminal extracellular domain of the bovine GABA_b α1 subunit with the bovine α6 subunit and expressed these chimeras in combination with the γ1 and γ2 GABA_b subunits in transfected mammalian cells. The expressed receptors in the cell membranes were characterized by displacement of either [3H]flunitrazepam or [3H]Ro 15-1788 with CLB 218 and methyl β-carboline-3-carboxylate. Channel integrity and function were studied by electrophysiological analysis. Our results will be discussed in this presentation. References (1) Pritchett, D.B. et al. (1989). Science 245, 1389-1392.

483.4

POSITIVE AND NEGATIVE REGULATORY ELEMENTS IN THE 5' FLANKING REGION OF THE MUSCLE NICOTINIC ACH RECEPTOR γ-SUBUNIT GENE. B.P. Gilmour and P.D. Gardner. Program in Molecular and Cellular Neurobiology, and the Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756.

ACHr expression is profoundly influenced during the development of skeletal muscle. Several lines of evidence suggest that this regulation is mediated in part at the level of transcription. Our laboratory is characterizing transcriptional control elements located in the 5' flanking regions of the ACHR subunit genes. Previously we have shown that 720 bp of 5' flanking DNA of the mouse muscle 5-consulubin gene is sufficient to direct cell-type-specific and developmentally regulated transcriptional repression in transiently transfected muscle cells. To further delineate the elements responsible for this regulated transcription, we have carried out deletion analysis of the 720 bp region. Deletion of a 172-bp region (nucleotides -526 to -355 relative to the transcription start site) resulted in a 5-fold increase in reporter gene expression. Placement of this 172-bp putative repressor region upstream of a heterologous promoter (e.g. SV40) resulted in a 5-fold decrease in reporter gene expression. Further deletion of the 5' flanking DNA (nucleotides -354 to -131 relative to the transcription start site) resulted in a 2-fold decrease in expression. These data suggest the presence of both negative and positive regulatory elements in the 5' flanking DNA.

483.5


Despite the high degree of homology between the human and the rat preproenkephalin (ppENK) promoters, (93% from +1 to -140, 50 to 605 beyond) expression of this gene varies between tissue and cell type, (La Gamma et al., Int. J. Dev Neurosci. 7:499, 1989). This raises the question, is it the cell background or the DNA sequence that confers this specificity? We have made chimeric ppENK-CAT promoter constructs extending to -497, -370, -249, and -152 base pairs from the start site of the rat gene (confirmed by sequencing and restriction analysis). These constructs were transfected into CV1 cells. Transfection efficiency was controlled by co-transfection with the pRL-TK (beta-galactosidase co-transfection. Basal expression was not detected in any of these constructs. Human ppENK was induced by treatments that increase intracellular cAMP ( Forskolin or TPA plus IBMK); similar treatments had no effect on any of the rat constructs. Although the CRE's of the human ppENK promoter are responsible for most of the cAMP inducibility of this gene, this appears not to be the case in the rat. We found five Single base pair differences in the region of CRE1 and 2 (Durkin et al., Neurosci. Abstr. 990). Sequence differences and/or cell-specific transcription factors, could generate complex patterns of gene expression. Supported by NSF #IBBS719872.

483.6


Tryptophan hydroxylase (EC 1.14.16.4) catalyzes the hydroxylation of tryptophan to form 5-hydroxytryptophan in the pathway of serotonin biosynthesis. As the major brain amine in the brain the serotonin pathway, it is important in maintenance of vascular homeostasis, intestinal motility, and central nervous system functions. Tryptophan hydroxylase, along with phenylalanine and tyrosine hydroxylases, comprise a family of aromatic amino acid hydroxylases that require tetrahydrobiopterin and molecular oxygen for the oxidation of the respective amino acids. The structural homology shared by these aromatic acid amino acid hydroxylases probably reflects their common origin in a single ancestral gene. Although the genes for human and rat phenylalanine and tyrosine hydroxylases have been isolated and characterized, only cDNAs for human phenylalanine hydroxylases have been described. We now report the isolation and characterization of genomic DNA for human tryptophan hydroxylase. The genomic DNA is intermediate in size to that encoding tyrosine and phenylalanine hydroxylases, spanning over 15 kb. A Msp I restriction fragment length polymorphism has been identified using a 5' genomic fragment as a probe, and its use for studies of possible involvement of tryptophan hydroxylase in human genetic disorders will be presented.
483.9

SPECIES AND REGIONAL DIFFERENCES IN THE EXPRESSION OF CELL-TYPE SPECIFIC ELEMENTS AT THE HUMAN AND RAT TYROSINE HYDROXYLASE GENE LOCI. G.T. Coker III, K.-Y. Gaudelius, M. Moffat*, and K.L. O'Malley. Dept. of Anatomy and Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

The expression of tyrosine hydroxylase (TH) is confined to different types of neuroendocrine cells. Using a transient assay system, we examined >10 kb of the human gene and 9.5 kb of sequencing the rat gene for DNA elements that confer cell-type specific expression. Results derived from the PC12 pheochromocytoma, LAN-1 neuroblastoma, and HepG2 hepatoma cell lines localized cell-type specific sequences for the rat and human genes. Surprisingly, these elements do not appear to be conserved in positive or negative across species. When plasmids containing DNA sequences >749 bp from the transcription start site of the rat gene were introduced into PC12 cells, 2 to 6 fold higher levels of expression were observed compared to the same fragments introduced into HepG2 cells. In contrast to the rat gene, analogous fragments of the human 5' flanking sequence failed to confer cell-type specific expression. However, when plasmids containing a thymidine kinase promoter and either orientation of a 700 bp 3' human gene fragment were introduced into PC12 and LAN-1 cells, we observed a 6.5-fold increase, respectively, over that observed for HepG2 cells. Deletions of this fragment led to significant activation of transcription, as much as 50-fold over control level. These data suggest the presence of positive and negative elements contributing to the tissue specific expression of tyrosine hydroxylase genes.

484.1


We have recently demonstrated that both SS and GHRH mRNA content in the hypothalamus of adult male rats vary in a manner consistent with the existence of an ultradian rhythm. However, the mechanism underlying this rhythm is unknown. Since previous investigations have demonstrated that GH can stimulate SS mRNA content, at least during a period of days, we tested the hypothesis that GH can stimulate SS mRNA content with a time course sufficiently rapid to account for the ultradian rhythm. Hypophysectomized adult male rats were fitted with jugular catheters, injected with either GH (1 ng) or vehicle and sacrificed at 2, 4 and 8 hr after injection (n=4 at each time).

Using in situ hybridization, we measured SS mRNA levels in individual neurons of the PVN and compared these levels among experimental groups. We report results (based on ~50 cells per animal) that SS mRNA content is increased at 2 and 4 hr after the GH injection and returns to control values by 8 hr.

Conclusions: Rapid feedback of GH on SS gene expression may underlie, at least in part, the ultradian rhythm in SS mRNA content in the hypothalamus of the adult male rat.

484.2


Somatostatin (SOM) mRNA decreases in rat hypoglossal nucleus (12H) from birth through postnatal day 14 (ROSEN J. 1:AJ91, 1989), after which time it disappears from 12H but remains in other brain nuclei, e.g. nucleus tractus solitarii (NTS). This tissue- and time-specific expression may be due to the presence of different trans-acting regulatory factors (i.e. proteins) in particular brain nuclei at different ages. Therefore, we performed gel-shift assays by incubating crude nuclear protein with 5'-labelled fragments of the SOM 5' flanking region. Resultant DNA-protein complexes were examined by polyacrylamide gel electrophoresis. To study time-dependent SOM expression, crude nuclear protein extracts from whole brainstem at ages 0, 7, 14, 21, and 28 days were assayed. To examine tissue-specific SOM expression, crude nuclear protein extracts prepared from adult rat 12H and NTS were used. In both sets of experiments, several specific DNA-protein complexes were identified as far as 750 bases upstream of transcription start site. These findings show that proteins isolated from brain tissue exhibit specific binding to the SOM promoter region. Significance of these DNA-protein interactions in tissue- and time-specific SOM expression in brain is unclear. (NIH HL38313, AR 81108)

484.3

HALOPERIDOL RAPIDLY INCREASES NEURONTENSIN mRNA LEVELS IN RAT NEOSTRIATUM. K.M. Merchant, M.A. Miller, E.A. Ashleigh* and D.M. Dorsa. GREEC, VA Medical Center and Dept. of Medicine and Pharmacology, University of Washington, Seattle, WA 98105.

Several recent studies have demonstrated that treatment of rats with antipsychotics such as haloperidol increases neurtensin (NT) immunoreactivity in the neostriatum. The purpose of the present work was to investigate the response of the NT/neuromedin N gene in striatal cells following acute and chronic treatment with haloperidol. Male Wistar rats were treated with a single dose of haloperidol (0.5 mg/kg, i.p.) and were sacrificed 1 hr later or b) multiple doses of haloperidol (0.5 mg/kg/d for 21 d, i.p.) and sacrificed 20-22 hr after the last treatment; controls were treated with the vehicle of the drug. In situ hybridization histochemistry using a 35S-labeled antiserotonin riboprobe was employed to determine the level and distribution of NT mRNA. A computer-assisted image analysis system was used to evaluate the data. In control brains labeled cells were observed in the stratum, nucleus accumbens, median forebrain bundle, olfactory tubercle and the septal nuclei. In anatomically matched striatal sections a sampling of the dorsolateral region showed that there was a dramatic increase in the number (control:42±13, haloperidol:154±30 unilaterally) as well as the optical density of hybridization-positive cells within 1 hr after a single dose of haloperidol (P<0.02, ANOVA). Such an increase was not observed in the dorsolateral striatum 20 hr after the chronic treatment. Thus haloperidol may acutely enhance the expression of the NT/neuromedin N gene in the neostriatum. (Supported by NS 20311).

484.4


The opioid peptides dynorphin (DYN) and enkephalin (ENK) are expressed differentially in dentate granule cells in that DYN is expressed at high levels in the basilar while basal expression of ENK is low. However, following hilar-lesion (HL) induced recurrent limbic seizures, all granule cells also express preproENK mRNA at high levels. In this study we examined preproDYN expression following HL-induced seizures to examine the regulation of expression of co-localized transmitters. Unilateral HL were placed stereotactically in ketamine-xylazine anesthetized male Sprague-Dawley rats. This procedure reliably produces bilateral electroencephalographic seizures within 90 min of lesion placement that recur for up to 10 hr thereafter. At selected times after HL, animals were killed and dentate gran/CA1, CA3, entorhinal and neo-cortical subfields dissected. Total RNA was isolated from these samples and the amount of preproDYN mRNA present in each individual sample was measured by nuclear protection analysis. In dentate gran/CA1 samples, preproDYN mRNA levels were found to increase 4-fold and 8-fold above control values by 6 hr and 12 hr post-HL respectively. PreproDYN mRNA levels returned to control values by 24 hr post-HL and were then suppressed 5-fold below control values at 96 hr post-HL. In situ hybridization analyses confirmed that these changes in preproDYN expression occurred in granule cells; additionally, these latter studies also revealed a small population of CA1 pyramidal neurons that were induced to express preproDYN by 48 hr post-HL. Preliminary results suggest that in entorhinal but not neocortex, preproDYN expression was also elevated during recurrent seizure. These findings contrast with those for ENK expression and suggest that different cellular mechanisms regulate expression of these two opioid peptide genes in hippocampal neurons. (NIMH 42074, 00801 (IDW); NIH NS 20748 (CMG)}

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
4.15
TRANSREGULATORY PROTEIN OF ADRENAL PREGNENOLONE: A NAIVEX.
B.L. Agarwal, J.D. DeCristofaro and E.P. La..

gamma, Peds & Neurobiol, SUNY at Stony Brook, NY 11794.

In the adult rat, hypoglycemic-induced transregulatory mechanisms only partially account for the increased levels of pregnenolone (ppENK) mRNA (Kanamatsu, FRAS 83:9245, 1986). Two days after transregulation, neonatal rats were made hypoglycemic at a time when functional synapses are not yet formed. Pups were treated with 20 U/kg of insulin or saline vehicle and sacrificed 24 hours later. Labeled mRNA in neurons of the hypothalamus was determined within the day and 3 of life (Lau, Dev Br Res 44:109, 1988). Controls received saline vehicle or were left unmanipulated. Hypoglycemia resulted in a 20-fold increase in adrenomedullary ppENK mRNA levels only in those insulin treated pups. To identify effects of hypoglycemia, independent of insulin treatment, intracellular glycopenia was produced by injecting adult rats with 2-deoxy-glucose (500 mg/kg). Similar to the insulin-hypoglycemia, there was an increase in adrenomedullary ppENK mRNA. We conclude that enkephalin biosynthesis as co-stored catecholamines appears to be induced by a transregulatory process. However, decreasing transregulatory activity by denervation or ganglionic blockage also increases ppENK mRNA, a paradox which remains unexplained. Supported by the March of Dimes Foundation.

4.17
DIFFERENTIAL EFFECTS OF ADRENAL AIDEON ON NPY mRNA LEVELS IN THE ARC, MEDIAL BULB AND MIGRATIONAL LOBE.
D.A. and B.J. Martin, Dept. of Foods and Nutrition, University of Georgia, Athens, GA 30602.

Neuropeptide Y (NPY) is a potent inducer of food intake. Its site of action is thought to be at the paraventricular nucleus (PVN) of the hypothalamus. Cell bodies of NPY nerve terminals in the PVN are located within the arcuate nucleus of the hypothalamus and within various nuclei of the brain stem. We have previously shown that adrenomedullectomy (ADX) decreases NPY mRNA levels in some regional brain areas (brain, hypothalamic, L.H). To determine if ADX decreases NPY mRNA levels in various areas, we examined the affect of ADX on NPY mRNA levels in the arcuate nucleus and brain stem. They were divided into three groups: 1) ADX, 2) ADX + corticosterone, or Sham. Days after surgery, tissues were taken and the total RNA isolated. Dot blots were made and hybridized with a cRNA probe to NPY mRNA. NPY mRNA was quantitated by densitometry. ADX decreased NPY mRNA levels in the arcuate nucleus by 50% (p<0.05). The decrease was reversed by corticosterone replacement. ADX had no affect on NPY mRNA levels in the brain stem. These results suggest that glucocorticoids may have a differential effect on the gene expression of NPY in the arcuate nucleus and brain stem. This implies that regulatory differences may exist between the arcuate nucleus and brain stem in the control of NPY levels in the PVN.

4.18
CHARACTERIZATION OF A NUCLEAR PROTEIN BINDING SITE IN THE 5'-FLANKING REGION OF THE MOUSE POMC GENE.
J.P. Bishop and M.M. Mouradian, Experimental Therapeutics Branch, NINDS/NIH, Bethesda, MD 20892.

Several protein binding sites have been identified in the 5'-flanking region of the mouse POMC gene using AT-GT cell extracts in exonuclease protection assays. In this study, the binding site located between -137 and -111 was further characterized because one of its sequence homology (TTGAGCCA) to AP-1 binding site (5 of 7 bases), and inducibility of POMC mRNA in these cells by phorbol esters. A double-stranded oligonucleotide probe containing a sequence of this was used in gel retardation experiments with AT-20 cell nuclear extract. A heat labile factor(s) exhibited specific binding to the probe which was abolished by a 50-fold excess of unlabeled homologous DNA, and was unaffected by oligos containing other known AP-1 sites. In the presence of this probe, a 1:1 complex was formed in the promoter region of the mouse POMC gene. This target is a target for an as yet unidentified nuclear protein. Whether binding characteristics different than those of authentic AP-1.

4.20
OXOTOCIN mRNA IS PRESENT IN THE RAT NEUROINTERMEDIATE LOBE.
Brooks, P.J. Lund, P.K., Caldwell, J.D., Jirkowski, G.F. and Pedersen, C.A. The Neurobiology Curriculum and the Departments of Physiology and Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, NC.

The mRNAs encoding vasopressin (AVP) and oxytocin (OXY) are synthesized in cells located in the posterior and supraoptic nuclei. At last year's Neuroscience meeting, two groups (Lightman et al., 142.5, McCabe et al., 141.2) presented evidence indicating the presence of AVP mRNA in the posterior pituitary. In the present experiment, we used a solution hybridization—ribonuclease protection assay to detect OXY mRNA in the rat neurointermediate lobe.

Pituitary glands were removed from lactating female rats and dissected into anterior (AL) and neurointermediate (NIL) lobes. Total RNA was isolated from the tissues and hybridized in solution with a 162 base 32P labeled cRNA probe derived from PGEoxyctc (plasmid provided by D. Tom Sherman, Univ. of Pittsburgh). A protected band corresponding to OXY mRNA was seen in RNA samples from the NILs, but not from the AL. This result indicates that, like AVP mRNA, OXY mRNA is also present in the rat NIL. Ongoing experiments are designed to determine whether NIL OXY mRNA may be present in axons from magnocellular OXY neurons.

4.40
NATURE AND DISTRIBUTION OF PREPROTACHYKININ mRNA IN HUMAN BASAL GANGLIA.
Center for Cell Biology, Sinai Hospital, and Wayne County Medical Examiner's Office, Detroit, MI 48235.

Preprotachykinin (PPT, i.e. substance P/neurokinin A- encoding) mRNAs in human basal ganglia tissues obtained at autopsy were quantitated using various human PPT cDNA clones isolated from basal ganglia (courtesy of T. Bonner, NHM) or hypothalamic cDNA libraries. The size of the mRNAs as determined by autoradiography was approximately 1.3 kb. This was somewhat larger than the previously reported size of 0.8 kb. This size difference was consistent with the presence of various PPT mRNAs encoding different combinations of tachykinin peptides in human basal ganglia. These differences were seen in a small number of cases. The quantitation of PPT mRNA was therefore undertaken using an incompletely spliced hypothalamic PPT cDNA clone containing both exon and intervening sequences.

4.41
ALTERED NEUROPEPTIDE GENE EXPRESSION IN EXPERIMENTAL DIABETES.
P.F. Pernghough, W.J. Smith and D.R. Tomlison.
Dept. of Pharmacology, St. Bartholomew's Medical College, London EC1M 6BQ, U.K.

The aim was to determine whether altered levels of substance P in diabetic rats could be gained by changes in gene expression of the precursor protein, preprotachykinin (PPT). Rats were studied 4 wk after induction of streptozotocin (50 mg/kg, i.p.) diabetes. Dorsal root (L4 and L5) and trigeminal ganglia were removed rapidly at death and total RNA isolated. Northern blots were prepared and probed using a (32P)-labelled 39-mer oligonucleotide probe complementary to PPT. Portions of sciatic nerve (1 cm) were removed from the same rats at death, snap frozen in liquid N2, substance P extracted (< 2h later), and substance P-like immunoreactivity (SPLI) was measured by radioimmunoassay. Sciatic nerve from diabetic rats contained much less SPLI (46.8±5.6 p.p.m. g nerve) than segments from control rats (87.4±12.5 [SEM] p.p.m. g, p<0.01). This was associated with markedly reduced levels of the PPT mRNA (relative to total RNA) in the dorsal root ganglia of the diabetic rats compared to control rats. PPT mRNA levels in trigeminal ganglia were, however, greatly increased in diabetic rats compared to control rats. These findings indicate that fundamental changes in gene expression for substance P occur in experimental diabetes.
485.1 CALCIUM IN DROSOPHILA NEURONS IN CULTURE. W. Dale Branton and Martha S. Rudnick*, Department of Physiology, University of Minnesota, Minneapolis, MN 55455.

Using the fluorescent [Ca] indicator fura 2, we have imaged [Ca] in neurons which were allowed to differentiate in culture from dissociated 4-5 h Drosophila embryos. We could consistently produce a rise in [Ca] levels in cell bodies, processes and growth cones by either rapid elevation of 

K+ or by application of toxins from scorpion venom (Tityus serrulatus). The scorpion toxins produce high rates of firing of action potentials in neurons via effects on voltage-dependent sodium channels. In either case the rise in [Ca] could be prevented by zero K+, 1 mM EGTA in the solution bathing the cells. The scorpion toxin-induced rise in [Ca] could also be prevented by TTX, which prevents the following action of the toxin by blocking sodium channels. Preliminary results indicate that pre-incubation of the cells with Plecuretoxin (PTX, Branton et al, J. Neurosci 7(12):4195-4200, 1987) can prevent virtually all the rise in [Ca] in the soma, processes and growth cones. PTX has been previously shown to block Ca++ currents in these cells measured under voltage clamp in whole cell patch configurations (Leung et al, Neuron 3:767-772, 1989). These findings with fura 2 are consistent with the previous findings and suggest the idea that the currents measured in whole cell patch are representative of currents in intact neurons. We are also investigating Heloene toxin (HoxT, Bowers et al PNAS 84:3920-3925, 1987), a selective blocker of non-inactivating Ca++ currents in these cells (Leung et al, Neuron 3:767-772, 1989), to determine if its action is spatially selective.


Single-cell ionized cytosolic calcium ([Ca]i) responses to depolarization (high K+), measured with fura-2, were potently inhibited by nimodipine (NMDK) in the highly differentiated clonal GH cell line. Individual cells which responded to high K+ were qualitatively similar to cell population responses: basal Ca++ (150 nM) increased to an initial high peak phase (over 400nM), followed by an extracellular Ca++-dependent plateau phase (200 nM), persisting more than 30 min in the absence of NMDK. A majority of cells (60%) also fired NIM sensitive spontaneous oscillatory Ca spikes, heterogenous with respect to both amplitude (fup to 200 nM) and frequency (up to 0.2 Hz). Some cells with stable Ca, developed spiking activity and/or reverted spontaneously. Oscillations were inhibited by depolarized conditions without drug. The high K+-induced sustained influx of 

Ca was completely eliminated by NIM, both in cell suspensions (10 nM), and in single cell determinations in monolayer (addition ≥50 nM). This correlated with potent NIM block of high K+-induced cell-associated Ca (IC50=0.2 nM). These data support both the physiological significance of sustained Ca influx to whole cell calcium metabolism, and the functional activity of NIM at nanomolar concentrations on plasma membrane Ca conductance.

485.3 DEPOLARIZATION-INDUCED CHANGES IN CYTOSOLIC FREE CALCIUM CONCENTRATION IN ISOLATED NERVE TERMINALS FROM RAT POSTERIOR PITUITARY. J.T. Russell, Section on Neuronal Secretory Systems, DN, NICHD, Bethesda, MD 20892.

Nerve endings (neurosecretosomes), isolated from rat posterior pituitaries were placed on protamine coated coverslips and perfused with balanced salt solutions. Cytosolic free calcium concentration in these nerve terminals was measured by monitoring the fluorescence of the calcium indicator, fura-2 using high resolution video microscopy. Intracellular calcium ion concentration rapidly rises in some of the terminals upon perfusion with solutions containing elevated potassium concentration (25 to 55 mM K+) or veratridine (6 μM). The initial rise is followed by a slow decline in [Ca2+]i to resting levels. The depolarization-induced increase in [Ca2+]i was inhibited by the presence of nimodipine, and u- 

conotoxin, two voltage-dependent calcium channel antagonists. Removal of sodium from the perfusion medium also caused a rapid rise in the intracellular calcium levels presumably due to the abolition of sodium-calcium exchange.

485.4 ACTIVITY-DEPENDENT INCREASES IN [Ca2+]i IN CULTURED RAT SEPTAL NEURONS. D. Blekasman, N.l. Harrison, J.D. Zacker, B. Wainer and B.J. Miller, Dept. of Pharmacological & Physiological Sciences and 1Dept. of Anesthesiology and Critical Care, Univ. of Chicago, Chicago, IL 60637.

We have begun to study the cellular processes which are involved in the buffering of physiological [Ca2+]i signals in CNS neurons. Single cell recordings using whole cell patch clamp recordings and fura-2 based microfluorometric measurements of [Ca2+]i, were made from cultured rat septal neurons at 25°C. Neuronal resting potentials were -55 to -65mV, input resistances 200-800MΩ and basal [Ca2+]i, values 130 ± 20nM (n=13). Neurons were held at -60V by constant current injection and action potentials (AP's) evoked by brief depolarizing current pulses. Trains of AP's elicited increases in [Ca2+]i that returned to basal values with approximately exponential kinetics (t=3s). High frequency (≥ 25Hz) trains of AP's produced larger increases in [Ca2+]i than low frequency (≤ 10Hz) trains for a given number of AP's, indicating a significant decline in the [Ca2+]i, between successive AP's at lower frequencies. The relationship between the number of AP's and [Ca2+]i, approached an asymptote at high [Ca2+]i, loads as previously observed in dorsal root ganglion cells (DRG) (S.A. Teyler and R.J. Miller, J. Physiol., in press). However, this asymptote was reached at 100-150 AP's compared to 20-60 AP's in DRG cells. Preliminary results suggest that both mitochondrial buffering and Na/Ca exchange mechanisms contribute to the recovery of the rise in [Ca2+]i following activity in cultured rat septal neurons.

485.5 SPATIAL AND TEMPORAL RESOLUTION OF CALCIUM TRANSENTS PRODUCED BY STIMULATION EFFECTS OF DIFFERENTIATING AMPHIBIAN SPINAL NEURONS. J Holland, R.J. Adams, T.J. Sejnowski*, and H.C. Spitzer, Dept. of Biology and Center for Molecular Genetics, University of California, San Diego, and Labor of the Computational Neurobiology, Salk Institute, La Jolla, CA.

Action potentials evoked from Xenopus spinal neurons exhibit a calcium dependence at initial stages of differentiation that shifts to a sodium dependence over a 24 hr period (Spitzer and Lamborghini, 1976). Therefore we expected to produce greater calcium influx at early stages of differentiation than during later periods of development. However, the intracellular calcium level attained upon stimulation is likely to be influenced by other factors such as the release of calcium from intracellular stores and calcium sequestration and buffering. These variables may also change over development.

Changes in the intracellular calcium levels produced by depolarization have been examined in intact (non-differentiated) cultured neurons using the calcium indicator, fluo-3 and high speed confocal image analysis. Elevations of intracellular free calcium in response to depolarization occur throughout the cell, most notably in the nucleus. The initial onset of increased indicator fluorescence is not detectably delayed in the nucleus relative to the cytosol under measurement conditions with a time resolution as fine as 2 ms. The fluorescence change was not detectably delayed in the nucleus relative to the cytosol under measurement conditions with a time resolution as fine as 2 ms. The initial fluorescence decay was fitted to first order kinetics with a rate constant of about 0.15 sec-1. After stimulation-evoked increase began to decline, slower elevations in fluorescence occurred. The contributions of calcium release from intracellular stores and calcium buffering to observed changes in intracellular calcium are considered. The effects of neuronal maturation on patterns of stimulus-evoked fluorescence will be described.

Supported by grants from the NIH to JH and NCS and from the NSF to TJS.


It is now well established that intracellular C2 stores play an important role in cellular regulation. C2 is stored within membranous organelles from which it can be released by second messengers. A large fraction of C2+-within these stores is bound to the Binding proteins which in the striated muscle and chick cerebellum include calquestrin. To further elucidate the nature of C2+-storing organelles in neurons, we have carried out a comparative analysis of the distribution of the IP3R and IP3-dependent C2 release in cerebellar Purkinje cells (PC).

As previously observed by us (Nature 342:192-195, 1989), immunogold labeling of PC revealed a widespread distribution of the IP3R throughout the smooth endoplasmic reticulum (sER), including tubules and cisternae present in dendritic spines and axon terminals. In addition, peculiar stacks of sER cisternae were frequently found near the cytoplasmic surface of the plasma membrane. Portions of these cisternae apposed to each other were particularly enriched in IP3R (for the concentration of the IP3R at these sites see also Sato et al, JCB in press), and appeared to be connected by thin-like elements of those connecting sER cisternae and tubules at muscle tracts.

Calquestrin immunoreactivity was concentrated selectively in a subset of the PC smooth surfaced tubular elements which are spines and axon terminals but was not enriched in the lumin of IMS receptor-rich stacks of sER cisternae. In addition, it was not detectable in the rough ER. These findings are consistent with the existence of structurally specialized subcompartments in C2+-storing organelles.
485.7

Plasma membrane inositol phospholipid-operated calcium channels have previously been found in lymphocytes and lens cells and are believed to be involved in endoplasmic reticulum calcium stores. We demonstrate in Xenopus oocytes that inositol 1,4,5-trisphosphate (IP3) activates a calcium influx (measured via a calcium-activated chloride current) which is antagonized by calcium with an IC50 of about 200nM. This conductance was insensitive to the TSCC antagonists verapamil and amiloride. Measurement of the conductance changes and reversal potential in the presence of calcium ions supports the existence of a native IP3-sensitive calcium channel (Capp3) in the oocyte. We find that the opening of this channel is accompanied by refilling of the IP3-sensitive calcium pool from the extracellular medium. We propose that a second messenger-operated calcium channel contribute to the refill of IP3-releasable intracellular calcium stores.

485.8
GENERATIONAL ACTALOSIDES (A1C0) EFFECT ON MITOCHONDRIAL Ca2+ UPTAKE. S. Koga & M.T. Ramirez. University of Chile; Faculty of Medicine; Institute of Experimental Medicine; Laboratory of Neurochemistry; Santiago 7-Chile.

It has shown in several models of experimental alcohols that A1C0 the first product of BOD oxidation reaches all organs, including several CNS areas all connected to metabolism of the toxic. The cell membrane is one of the target structure of deleterious effect; in neuronal tissue affects transport, depolarization, nervous cell, etc. and these processes linked to Ca2+ transmembrane transport. Our Institute has created an experimental model whose rate over seven generations were exposed to A1C0 along their lives from embryo to adult including crossing (Koga et al. since 1987).

Adult albino Wistar rats & Normal and A1C0/R rats these last were injected daily 200 mg/kg rat A1C0 ip., during their life: intraterine, lactation (by mother injection), immature and adult age, during 7 generations. Five mitochondrial Ca2+ areas were studied: brain cortex, hypothalamus, hypophysis, cerebellum & midbrain. Site I mitochondrial electron transport and Ca2+ influx were studied with a polarigraph and "dip box" selective electrode with 4.5 ml chamber with adequate respiratory medium. Results showed that Ca2+ uptake in A1C0/R rats compared with normal, particularly when hypothalamus, hypophysis and cerebellum were studied.

485.9
MOTILYSIS OF THE CAGED Ca2+ Chelator DIAZO-4 RAPIDLY INCREASES Ca2+ ACCUMULATION AND Ca2+ CHANNEL INACTIVATION IN APOLYSIS NEURONES. M.W. Fryer and R.S. Zuckler. Dept. of Mol. & Cell Biology, Univ. of Calif., Berkeley, CA 94720.

Ca2+ mediated Ca2+ channel inactivation in Aplysia neurones is seen as either a decline of Ca2+ current during long depolarizing pulses or as the loss of peak current in a test pulse (P2) following a pre-pulse (P1). We used the photolabile Ca2+ chelator diazo-4 (Adam et al., J. Am. Chem. Soc., 111, 7957, 1989) to generate rapid decreases in intracellular free Ca2+ ([Ca2+]i) under both sets of conditions. Left upper quadrant neurones from the abdominal ganglion were injected with a diazo-4/GCl mixture to a final diazo-4 concentration of 2-5M. Then a brief intense light flash was given during a long depolarizing step the decline of Ca2+ current was markedly slower than the control. Separation of the overlay current relaxations was apparent after approximately 1ms. In paired-pulse experiments, a light flash given in the inter-pulse interval increased the Ca2+ peak current ratio and hence accelerated the recovery from inactivation. The results show that diazo-4 may be used to rapidly buffer the high local [Ca2+]i that is present near Ca2+ channels during and after activity. The kinetics of such changes are somewhat more rapid than expected from Ca2+-dependent enzymatic mechanisms of inactivation development and recovery. Supported by NIH Grant NS 13114 and NHMRI (Aust.) C.J. Martin fellowship to M.F.

485.10

It has been suggested that extracellular Ca2+ current channels are modulated intracellularly both by CaM-P and Ca2+-dependent processes. Using an internal perfusion technique, we have isolated Xenopus laevis neurons, we have identified a Na+ current activated at the resting potential by a combination of CaM-P and theophylline, which appears in the absence of any effect on the voltage dependent calcium (Ic) or potassium (Ig) currents. The magnitude of this current can be reversibly reduced if ATP is not added to the internal perfusate. Suggesting that CaM-P-dependent, phosphorylation may selectively modulate this current over Ic and Ig. In contrast, Ig is modulated by internal calcium. Inactivation, as measured by a double-pulse paradigm, is reversibly enhanced by decreasing the concentration of intracellular calcium buffer (EGTA), and the long-term disappearance of Ig (washout) is increased with a larger calcium load. Maximal current currents, stimulated every six seconds with 500ms pulses, increased the rate of washout over four times, as compared to cells monitored with 20ms pulses every minute. We hope to further delineate calcium's role in regulating Ic using photorelease of caged Ca2+ to produce sudden increases in intracellular Ca2+.

485.11
EXOGENOUSLY-ADDED ATP PROMOTES 35Ca INFLUX IN PC-3M CELLS. D.A. Olechansky, M.L. Koenig, M.A. Jackson, and J.B. Trepel. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307 and Lab of Pathology, NCM, Bethesda, MD 20892.

It has been reported recently that addition of ATP to the extracellular medium can transiently increase cytosolic Ca2+ concentrations in cultured neuronal cells (J. Neurochem., 39:293-301) and is associated with synaptic potentiation in hippocampal slices (Brain Res., 491:36-9). To further characterize the effect of exogenous ATP on Ca2+ homeostasis, we have studied PC-3M cells, a prostate tumor cell line which demonstrates a large transient increase in free intracellular Ca2+ in response to added ATP. We now show that at least part of the ATP-dependent increase in intracellular Ca2+ levels in PC-3M cells can be attributed to an enhanced influx of Ca2+. The ATP-stimulated uptake of 35Ca is saturable, does not appear to be voltage-gated, and is relatively slow (t1/2=2 min). The response is dose-dependent with maximal influx at an added ATP concentration of 5mM. influx is markedly reduced at higher concentrations of the purine. Preliminary experiments with verapamil and a number of inorganic Ca channel antagonists suggest that the influx occurs via an ion channel. Dihydropyridine channel blockers appear to be unable to block the ATP-stimulated uptake of Ca2+. These results indicate that ATP is able to alter Ca2+ homeostasis by a direct effect on a non-voltage gated Ca2+ channel.
486.1 CALCIUM CHANNEL REOPENINGS AT RESTING MEMBRANE POTENTIALS FOLLOWING PRIOR DEPOLARIZATION. P. A. Sleigher and J. B. Langston, Graduate Neurosciences Program, Univ. of Calif. Med. School, San Francisco, CA 94143.

Neurotransmitter release depends critically on Ca influx through voltage-dependent Ca channels. Although Ca current is inhibited by a wide variety of substances, mechanisms for potentiation of Ca current are less well known. Ulna Ca currents were recorded with 90 mM Ba in the pipette and a NaCl solution in the bath. Granule cells possess a single class of dihydropyridine-sensitive Ca channels (n=10) with a voltage dependence of V_{1/2} = 6.9 mV. With depolarizations positive to -30 mV (V_h = 100 mV), depolarizations to -0 mV for 100 ms elicited brief openings with a mean open lifetime of -0.5 ms. Following the termination of the voltage step, Ca channels close with a rate too fast to be detected at -70 mV. By contrast, two types of Ca channel activities are observed following termination of very prolonged prepulses (>50 mV): channels that remain open at the end of the prepulse and channels that close, but reopen briefly after a delay. We analyzed the kinetics of Ca channels that were open following the prepulse. Distribution of open time durations for all openings following the prepulse was fit by a single exponential with a t_{1/2} = 4.5 ms. Distribution of open time was fit by a single exponential with a t_{1/2} = 7.7 ms. With shorter, positive prepulses (<25 ms), fewer reopenings are produced and the time constant for the first latency histogram was faster. Thus, Ca channel reopenings depend on both the amplitude and duration of prepulse. These results are consistent with Ca channels returning from an inactivated or blocked state and suggest that following a train of action potentials, the opening of Ca channels at membrane potentials far from the equilibrium potential for Ca would produce a large component of Ca influx.

486.3 MONOClonAL antibOdIES To THE \( \mu \)-COnToXIN-SENSITIVE CALCIum CHANNEL FROM RABBIT Brain MEMBrANEs Junji Sakamoto, K.K. Stang* and K.P. Campbell. Howard Hughes Medical Institute and Dept. of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242.

The high affinity \( \mu \)-conotoxin receptor of the calcium channel from rabbit brain membranes has been characterized using monoclonal antibodies. Mice were initially injected with isolated rabbit brain membranes and boosted with heparin-agarose eluate from rabbit brain membranes, which was enriched in the \( \mu \)-conotoxin receptor. Hybridoma supernatants were screened for immunoprecipitation of \( \mu \)-conotoxin binding activity. Monoclonal antibodies from hybridoma culture supernatants were preincubated with goat anti-mouse \( \mu \)-sarcocecid botytoxin (GAM) beads to form monoclonal antibody-GAM beads. These beads were then tested for their ability to immunoprecipitate the \( \mu \)-conotoxin-prelabelled receptor from CHAPS-solubilized rabbit brain membranes. These monoclonal antibodies were found to specifically immunoprecipitate the \( \mu \)-conotoxin-labeled receptor. The \( \mu \)-conotoxin-sensitive calcium channel has been isolated from total rabbit brain membranes using a combination of heparin-agarose chromatography, sucrose density centrifugation and WGA-sarcocecid botytoxin chromatography. The monoclonal antibodies specifically precipitating \( \mu \)-conotoxin binding activity are being used to characterize and identify the subunit composition of the \( \mu \)-conotoxin-sensitive calcium channel isolated from rabbit brain membranes. K.P. Campbell is an Investigator of the Howard Hughes Medical Institute.

486.4 Isolation of a Rabbit Brain Transcript with Homology to the \( \beta \) Subunit of the Skeletal Muscle Dihydropyridine Sensitive Calcium Channel. M. Pragnell, S.D. Jay, C.J. Levitte and K.P. Campbell. Howard Hughes Medical Institute, Neurosciences Program and Dept. of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242.

The dihydropyridine sensitive calcium channel, enriched in rabbit skeletal muscle T-tubules, contains four subunits (\( \alpha_1,\beta,\gamma \) and \( \delta \)). All of these subunits have been recently cloned. Sequence information suggests that \( \alpha_1 \) is the principal transmembrane subunit which contains the pore of the ion channel. The cDNA sequence of the \( \beta \) subunit predicts numerous conserved phosphorylation sites (Ruth et al.: Science 245:111) consistent with in vitro studies suggesting a regulatory role. We have isolated a cDNA for the \( \gamma \) subunit (Jay et al.: Science 248:400) and the \( \beta \) subunit. cDNA probes derived from the skeletal muscle \( \beta \) subunit message have been shown to crosshybridize with a 3 kb mRNA species in rabbit brain. This is significantly larger than the 1.8 kb message observed in skeletal muscle. We have screened a rabbit brain cDNA library with probes made from the skeletal muscle \( \beta \) subunit cDNA clone. A 0.5 kb and a 2.2 kb insert were isolated that crosshybridized with the skeletal muscle \( \beta \) sequence. These inserts were then used as probes to rescreen the library and isolate full length cDNA clones. Following completion of sequence analysis a comparison will be made between the predicted structure of the encoded protein and that of the skeletal muscle \( \beta \) subunit. This will test the hypothesis that a homologous brain protein exists which has a calcium channel regulatory function analogous to that proposed for the skeletal muscle \( \beta \) subunit. K.P. Campbell is an investigator of the Howard Hughes Medical Institute.

486.5 PRIMARY STRUCTURE AND EXPRESSION OF THE RAT BRAIN CLASS-A CALCIUM CHANNEL. T.Y. Starr, W.A. Pyrstya* and TERRY P. Snutch. Biotechnology Laboratory, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5.

We have isolated a number of rat brain cDNAs which are homologous to the \( \alpha_1 \) subunit of the dihydropyridine receptor/Ca channel of rabbit skeletal muscle and heart. Preliminary experiments show that at least four distinct classes of \( \alpha_1 \) channel genes are expressed in rat brain. Currently, we are investigating the functional and molecular relationships between the different neuronal Ca channel family members. Using Northern blot analysis, rat brain clone A cDNAs (rBa) hybridize to two major transcripts of approx. 8.3 and 8.8 kilobases (kb). Partial sequence analysis of 20 clones of clone A cDNAs indicates that these clones fall into two subfamilies that differ at their 3′ ends. One class A clone, rBa-126, has been entirely sequenced and contains a single repeat unit. The deduced amino acid structure of rBa-126 shows that it contains four repeated homology domains, each containing six putative transmembrane segments. The deduced amino acid sequence is 55% identical to the cardiac Ca channel. In addition, rBa-126 possesses two large hydrophobic domains not related to sequences found in previously cloned 470 amino acid segment that separates domains II and III. The second is an approx. 600 amino acid region which follows domain IV. The significant structural differences between closed Ca channels suggest the possibility that functional differences may also exist.

486.6 PRIMARY STRUCTURE AND EXPRESSION OF THE RAT BRAIN CLASS-B CALCIUM CHANNEL. S.J. Dubel and T.P. Snutch. Biotechnology Laboratory, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5.

We have found that rat brain expresses a family of genes (Class A, B, C and D) that are related to the \( \alpha_1 \) subunit of skeletal muscle and cardiac Ca channels (Snutch et al., PNAS 90:1990). ANorthern blot analysis reveals that a set of cDNAs designated rat brain class B (rBB) hybridize to a brain transcript of approx. 10 kilobases (kb). A series of overlapping cDNAs were isolated, sequenced and found to contain a single open reading frame of 6.8 kb. The predicted rBB peptide is most closely related to the rat brain class B Ca channel sequence (with greater than 90% identity in the five repeated homology segments; see abstract by Starr et al.). However, homology between the class A and B Ca channel peptides differs significantly in two putative cytoplasmic domains. The first of these domains is located between homology repeats II and III (approx. 430 amino acids in rBB, while the second follows repeat IV (approx. 650 residues). In both the class A and class B peptides these two regions contain a number of potential CAMP-dependent phosphorylation sites. Studies are now underway to examine the temporal and spatial expression pattern of the rBB gene in the rat CNS, and to express a full-length rBB cDNA in a number of test systems.
468.7

ISOLATION OF cDNA CLONES FOR α1 SUBUNIT ISOFORMS OF THE L-TYPE CALCIUM CHANNEL FROM MOUSE BRAIN. W. L. Ma* and M. L. Usher. Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109.

The α1 subunit is the major transmembrane component of the L-type calcium channel. Furthermore, it contains the binding site for dihydropyridines and has been proposed to form the ionic pore of the channel. In order to characterize the neutral expression of the α1 subunit we have used a synthetic oligonucleotide complementary to the sixth transmembrane segment of the third repeat of the α1 subunit of the rabbit skeletal muscle calcium channel. cDNA clones related to the rabbit α1 skeleton sequence were isolated from mouse brain (DNA libraries. Two clones of cDNA clones were obtained. The first class of cDNA clones hybridizes to messenger RNA and 14 kb in mouse brain and appear to code for the mouse cardiac form of the α1 subunit. This cDNA codes for a protein that is 98% identical in amino acid sequence to the rabbit cardiac α1 subunit. The second class of cDNA clones contains many differences in nucleotide sequence from both the cardiac and skeletal sequences. Sequence comparisons suggest that this class of cDNA results from a transcription of a novel α1 subunit genomic sequence.

468.8

SINGLE CHANNEL PROPERTIES OF Ca2+ CHANNELS EXPRESSION FROM RAT BRAIN mRNA IN Xenopus OOCYTES. Lin, L.W. and Linde, R. Dept. Physiology and Biophysics, NYU Med. Ctr, NY 10016.

Calcium channels expressed from rat brain mRNA in Xenopus oocytes have been shown to be different from L, N and T types in that this calcium current is activated by high voltages, with little inactivation, and is insensitive to dihydropyridine or Ca antagonists (Dobrowski, Pajonk, and Sale, 1985). We have shown (1) that this current can be blocked by spider toxin (Lin et al., PNAS, in press, 1990) (FTX) from Agelenopsis atra (Lillenas et al., PNAS, 86, 1689; Cherksey et al., Soc. Neurosci., 1989, 15, 662). From the binding of the toxin is dependent upon the concentration of divalent cations. In order to further characterize this current, single channel recordings were obtained from cell patches, using an intracellular electrode and a computer membrane potential. The patch was clamped at -100 mV and 40 msec pulses were used to activate voltage sensitive calcium channels. With 70 mM BaCl2 and 1 μM TTX in the patch pipette, an inward unitary current of 0.58 pA was recorded at -20 mV and its amplitude decreased with further depolarizations. I/V plot of this unitary current, ranging from -40 mV to 0 mV, gives a slope conductance of 13 pS. In addition, in some patches, we observed a large unitary current with a slope conductance of 20 pS. Both unitary currents exhibited different sensitivities to depolarizations. For example, in one patch, the large unitary current was observed in 30% of the traces at 0 mV whereas the small unitary current in the same patch appeared in more than 65% of the traces at 20 mV. Both unitary currents appear throughout the duration of depolarizing pulses, suggesting a non-inactivating current similar to the macroscopic current recorded in intracellular recordings. We are currently under investigation. (Supported by Fidia Research Foundation and NIH grant NS13742 to R. Llinas, GM26976 to B. Rudy.)

468.9

EXPRESSION OF A RECOMBINANT OMEGA-CONOTOXIN IN YEAST. M.W. McLane*, B.A. Lampel*, D. Shields*, A. Salama* and M.M.S. Loel. 1st. Citia, Wilmington, DE 19897, and 2nd. Dept. of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, NY 10461.

Omeagaa-conotoxins are potent blockers of N-, Ca2+ channels within various species. Several naturally occurring variants have been isolated from the venom of Conus Snails. These are particularly small peptides, containing four to five residues, which are highly homologous, and are predicted to have a rigid structure conserved by 3 intramolecular disulfide bridges. This paper describes the expression of one of these native protease-resistant, binding and secretion in yeast, which results in the production of a biologically active molecule. A synthetic oligonucleotide encoding a variant of omega-conotoxin was fused to the pro-alpha-factor sequence. Yeast strains, transformed with the recombinant pro-alpha-protoxin construct, produced a major low molecular weight peptide. The amino acid sequence is being determined from a reduced and carboxymethylated fraction. Disulfide bridge assignment will be determined in the native yeast product after purification by reverse phase HPLC. The biological activity of the recombinant conotoxin will be initially determined by a toxicity assay in goldfish, and further characterized by inhibition of 125I-conotoxin (G6A) to rat brain membranes.

468.11


We have identified ryanodine binding proteins in cells of the avian central nervous system. Monoclonal antibodies against avian skeletal muscle sarcoplasmic reticulum fast protein were used to localize and isolate the brain protein. Neuronal ryanodine binding protein-like immunoreactivity was found on components of the internal membrane system of cerebellar Purkinje neurons. The subcellular distribution of the immunoreactive protein was established using a monoclonal antibody of thick sections, immuno-EM of probed tissue labeled semi-thick sections and by immunogold labeling of ultrathin cryosections. The ryanodine binding protein-like immunoreactivity was found in intracellular structures of the perikarya, the dendritic arbor and the axon. Ryanoide binding proteins were not found in dentate spines. Double immunofluorescent experiments, comparing the distributions of the ryanodine binding protein to the IP3 receptor and to calbindin, revealed differences in cytoplasmic distributions. For example, the IP3 receptor immunoreactivity is prominent in the KER and dentate spines (C. Murphy et al., Nature 342:592-195,1989) while the ryanodine binding protein appears absent in spines and is not distributed throughout the KER. Immunofluorescent experiments reveal that cerebellar ryanodine binding proteins have a native molecular weight of ~2,000 kD and are composed of two high molecular weight (~500 kD) polypeptide subunits. A comparable high molecular weight subunit was observed in the remainder of the brain. If the ryanodine binding proteins in muscle and nerve are similar in function, then the neuronal proteins may participate in release calcium from intracellular stores that are distinct from those of the recently characterized IP3-activated calcium channel.

468.12


Ryanodine, at low nanomolar concentrations applied to neurons in vitro, inhibits the release of calcium from caffeine-sensitive intracellular sites that appear to be localized to endoplasmic reticulum. In analogy to muscle sarcoplasmic reticulum, inhibition of neural tissues may be mediated through high affinity ryanodine binding sites. To investigate whether specific [3H]ryanodine binding sites are present on CNS tissues, we are conducting a series of studies using standard radioligand binding methods. Optimal binding was obtained at pH 8.0 in the presence of 500 mM ATP, 100 mM Ca, and 1.0 mM KCl. [3H]Ryanodine binding was linear in membrane protein concentrations ranging from 0.02 to 0.13 mg. In association experiments, equilibrium was reached within 15 min at 37°C and levels of [3H]ryanodine binding were stable for at least 2 hr. Kd values for P1 (nuclear), P2 (mitochondrial), and the (microsomal) fractions derived from saturation experiments were comparable at about 5 nM. The number of [3H]ryanodine binding sites in P2 fractions was approximately 4-fold greater than that in P1 or P3 fractions. [3H]Ryanodine may be binding to sites that mediate intracellular calcium release. (Supported by MRC of Canada.)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
CHARACTERIZATION OF A PUTATIVE INSET CNS CALCIUM CHANNEL, USING THE PHENYLALKYLAMINE [H]+/VERAPAMIL. J.W. Rees* and D.B. Sattelle. APCR Laboratory of Molecular Signalling, Department of Zoology, Downing Street, Cambridge CB2 3EJ, U.K. Spon: Brain Research Association

Membranes prepared from the CNS of the cockroach, Periplaneta americana, have been found to contain saturable, specific phenylalkylamine binding site, based on studies using [14]H]/verapamil. Scatchard analysis has revealed a single population of binding sites with a dissociation constant (Kd) of 57 ± 5 nM (mean ± SD, n=3) and a binding capacity (Bmax.) of 70 ± 20 pmol/g brain dry weight, per mg membrane protein (mean ± SD, n=3). Hill plots of the data yield a Hill coefficient of 0.96 ± 0.02 (mean ± SD, n=3) that does not depart significantly from unity, indicating the absence of cooperativity. Direct displacement studies using a range of putative calcium channel ligands reveal that the pharmacological profile of this [14]H]/verapamil receptor in cockroach CNS membranes differs from that of the well-documented vertebrate phenylalkylamine binding receptor that is a component of an L-type calcium channel. In particular, the insect CNS phenylalkylamine receptor is dihydropyridine-insensitive, in contrast to the finding for the corresponding site in vertebrates which is dihydropyridine-sensitive. These data indicate the presence of a putative calcium channel in the insect CNS that is not readily classified using pharmacological criteria established for vertebrate tissues.

CATECHOLAMINES VI


Previous studies from this laboratory have demonstrated a topographic relationship between projection neurons of the locus coeruleus (LC) and target regions of the neocortex and subcortical visual structures within the rat brain. The present investigation was conducted to examine the distribution of LC neurons that project to the ventrobasal (VB) nucleus of thalamus and the barreelfield region of the somatosensory cortex. An additional goal of the study was to determine if regions along the same sensory pathway receive collateral inputs from the same LC neurons. Long-Evans hooded rats (275-400 g) received unilateral pressure injections (1.0 ml) of various combinations of green and red fluorescent latex (rhodamine) microspheres into the barreelfield area of the somatosensory cortex and the VB nucleus. Coronal sections (80-100 um) through the LC were examined by fluorescent microscopy and the distribution of retrogradely labeled cells was recorded. LC neurons labeled from barreelfield injections were primarily located throughout the rostrocaudal dimension of the ipsilateral nucleus. By contrast, neurons projecting to VB thalamus were distributed bilaterally with a slight ipsilateral predominance. In the coronal plane, coeruleo-cortical projection neurons tended to be localized within the dorsal half of the LC, while cells projecting to VB thalamus had a tendency to be displaced more ventrally. Cells which were double-labeled were concentrated in the region of the ipsilateral nucleus where the subjects of cortical and thalamic projection neurons overlapped. In summary, the distribution of retrogradely labeled cells observed here suggests a rough topographic ordering of LC neurons with respect to somatosensory projection sites. Furthermore, the presence of double-labeled cells suggests that individual LC neurons may simultaneously influence the sequential processing of information at different levels within the different somatosensory pathway. (Supported by AFOSR 87-0138 and by an award from the Klingenstein Foundation to BDW)

EFFECTS OF VAGOTOMY ON THE Firing PATTERNS IN LOCUS COERULEUS NEURONS. C.L. Williams, E.J. Brea, S.E. Krahl, R.A. Jensen, and D.C. Smith, Department of Psychology, Southern Illinois University, Carbondale, IL 62901.

This study investigated the role played by peripheral factors in the regulation of maintained discharge of locus coeruleus (LC) neurons. The response characteristics of these cells can be affected by systemic administration of substances which do not freely enter the brain. For instance, Holdener and Jensen (Br. Res. 417/106, 1987) found a small increase in spontaneous activity of LC cells following peripheral administration of 8.2 mg/kg 4-OH amphetamine, and an increase in maintained discharge with 10.0 mg/kg of epinephrine. The activity of these cells can also be affected by surgical interventions which remove some of the peripheral neural input to the CNS. For example, Svensson and Thoren (Br. Res., 172/174, 1979) showed that increasing blood volume alters the spontaneous activity of LC cells, and this effect was abolished by cervical vagotomy.

In the present study, we designed to examine the effects of vagotomy on the response characteristics and maintained discharge of neurons in the LC. Complete subdiaphragmatic vagotomy was performed on Sprague-Dawley rats 2 weeks prior to all testing. Animals were maintained in cages and activity units were used in normal and vagotomized rats under urethane anesthesia with tunstung microelectrodes. In the vagotomized animals, we found a loss of spontaneous activity in some LC cells, as well as an altered response pattern to noxious external stimuli when compared to normal rats. These data suggest that peripheral factors transmitted to the brain via the vagus nerve play an important role in the modulation of the neuronal discharge patterns of CNS catecholamine systems. (Supported in part by NIH 1 T32 MH-18662 to E.J.B.)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

THURSDAY PM

1176
CATECHOLAMINES

hemodynamic

temporally

destined

rates

NITROPRUSSIDE

shorten

alpha-helical

analysis

THURSDAY

neurons

results

produced,

combined

CRF9-41

LOCUS

forebrain

infusing

EFFECTS OF LOCUS COERULEUS (LC) INACTIVATION ON EEG ACTIVITY IN NEOCORTEX AND HIPPOCAMPUSS. S.L. Poole, M.E. Page, C.W. Bertidge, and R.J. Valantion. Dept. Psychiatry (W-003), DCSO, La Jolla, CA $92093; (1) Dept. Mental Health Sci., Hahnemann Univ., Philadelphia, PA 19102.

The hypothesis that the noradrenergic neurons of the LC can influence EEG activity was investigated by inactivating these neurons in halothane-anesthetized rats while simultaneously recording neocortical (EGoG) and hippocampal (EGOG) EEG activity. LC inactivation caused a reduction of the P1 wave of the monophasic P wave, and a reduction of the slow-wave EEG activity. In addition, beta oscillatory activity was reduced by infusing the alpha-2 noradrenergic agonist clonidine into LC. Incomplete infusions of LC neuronal activity were produced by very low dose microelectrode recordings, were followed by high-amplitude, low-frequency EEG activity and mixed frequency EEG activity. Bilateral clonidine infusions induced a nearly complete blockade of foot-pinch elicited EEG activity in this preparation. This blockade was potentiated by systemic or ICV clonidine. These results suggest that the LC may affect noradrenergic neuronal activity in the neocortex and hippocampus. The present data complement our observations that LC activation in this preparation produces EEG desynchronization and EOG theta activity (Bertidge et al., this meeting).

STRESS-ELICITED ACTIVATION OF THE LOCUS COERULEUS (LC) BY NITROPRUSSIDE IS ASSOCIATED WITH EOG COREGULATORS OF AROUSAL. M.E. Page and R.J. Valantion. Dept. of Menal Health Sciences, Division of Behavioral Neurobiology, Hahnemann University, Philadelphia, PA 19102.

Stressors have been shown to increase spontaneous discharge rates of LC neurons (Svenson, 1987). Recently, hemodynamic stress responses to isometric handgrip (Nitratoprusside infusion) was found to increase LC discharge; this activation required endogenous cotictorip-increasing factor (CPRF), suggesting that LC activation during stress is mediated by CPRF. It was hypothesized that one function of LC activation is to increase or maintain arousal by hemodynamic stress. The effect of nitroprusside on cortical and hippocampal EEG and LC spontaneous discharge were investigated in halothane-anesthetized rats. A hyperactive EEG was produced by infusing the nitroprusside at 10µg/20umL/min (15 min) increased LC spontaneous discharge rate 31 ± 5% (N=9). The increases in LC discharge were temporally associated with increases indicative of arousal, i.e., low amplitude, cortical desynchronization and onset of hippocampal theta rhythm. To elucidate the role of CPRF in stress-induced EEG activation, a CPRF antagonist, alpha-helical CRF9-41 (150 µg) may delay the onset, and shorten the duration of EEG activation. This work was supported by PHS Grants MH40008 and MH42796.


We previously reported that LC neurons extended processes 200-500 µm into two pericortical zones which we designated the rostralmedial and caudal juxtaparaportal pericortical regions. EM-immunocytochemical analysis of these processes demonstrated that virtually all of these processes are dendrites which are heavily targeted by several morphologically distinct classes of extrinsic synapses. These experiments suggest that the pericortical orientation of LC neurons is a characteristic of many or only a subset of LC neurons. To address these questions we are analyzing the dendritic arbors of LC neurons impaled and filled with biocytin in horizontal brainstem slices.

Results to date suggest that (1) most LC neurons have pericortical dendrites: (2) the longest of these dendrites extend into either the rostralmedial or caudal juxtaparaportal pericortical regions, or both; and (3) neurons in all parts of LC sampled exhibit this preferential dendritic organization.

These results suggest that many LC neurons receive significant afferent inputs on extracellular changes which act on one or both pericortical zones. EM double labeling methods are necessary to determine which afferents to pericortical regions terminate on LC dendrites, pericortical neurons or both. (PHS Grant NS24698).

EFFECTS OF LOCUS COERULEUS ACTIVATION ON EEG ACTIVITY IN NEOCORTEX AND HIPPOCAMPUSS. C.W. Bertidge and S.L. Poole. Dept. Psychiatry, Univ. of Calif., San Diego, CA 92093.

The hypothesis that noradrenergic locus coeruleus (LC) neurons participate in forebrain activation as measured by EEG was examined. In halothane-anesthetized rats, small infusions (75-150 nL) of the noradrenergic agonist, carbachol, were used to reversibly activate LC neurons while simultaneous recordings of LC neurons verified this activation. Power spectrum analyses of frontal neocortical and hippocampal EEG were performed. In 11 animals in which histologically verified peri-LC infusions activated the LC, the findings were: 1) LC activation was followed, within 20 to 30 sec, by a shift from low-frequency high-amplitude to high-frequency activity in neocortex and hippocampus and the appearance of intense theta rhythm in the hippocampus; 2) In neocortex, absolute power of all frequency bands decreased; the largest decrease was observed in the 0.8-3.0 Hz bands; 3) Relative power increased in the 25.0-50.0 Hz and decreased in the 0.8-3.0 Hz bands; 4) hippocampal relative power increased in the 3.0-7.0 Hz and decreased in the 0.8-3.0 Hz bands; 5) infusions that did not activate LC neurons (e.g., placed 1 mm dorsal or ventral to LC), had no effects on forebrain EEG activity; 6) EEG patterns returned to baseline with about the same time course as the recovery of LC activity (10-20 min). These observations indicate that LC activation is the crucial mediating event for these infusion-induced changes in forebrain EEG. Supported by the U.S. Air Force (OSR) and the MacArthur Foundation.

EFFECTS OF THE 5-HT REUPTAKE INHIBITOR FLUVOXAMINE ON ANXIETY INDUCED BY ECT. D.S. Charney, G.R. Heninger, S.W. Woods, Dept. of Psychiatry, Yale U. Sch. of Med., 34 Park St., New Haven, CT 06508.

The alpha-2 adrenergic receptor antagonist yohimbine (YOH) produces robust anxiogenic effects in panic disorder (PD) patients. This study investigated the effects of chronic treatment with the 5-HT reuptake inhibitor fluvoxamine (FLUV) on YOH-induced anxiety in PD patients. Fifteen patients with DSM III-R PD completed a double-blind placebo-controlled study of FLUV. YOH (0.4 mg/kg IV) and placebo challenges were performed during a three week drug-free period and again after nine weeks of treatment. Within challenge measurements included visual analog scale (VAS) anxiety, DSM III-R somatic symptoms, blood pressure and heart rate, plasma cortisol (CORL), and 3-methoxy-4-hydroxy-phenylglycol (MHPG). RESULTS: Six of 8 patients responded to FLUV, as compared to 7 of 9 controls. Patients had a greater reduction in the net YOH VAS anxiety response than placebo did, (with FLUV, 48.8±47.9 mm to 11.8±44.8, p<0.06; with placebo, 41.9±20.9 to 40.7±36.8; NS; compare FLUV and placebo, p<0.04). FLUV but not placebo reduced somatic symptoms suggestive of YOH. Baseline and peak MHPG levels after FLUV did not differ from placebo compared to placebo treated patients. CORL levels in both groups rose after YOH but there was no significant difference between the two groups. Blood pressure and heart rate after YOH were not affected by FLUV compared to placebo treated patients. DISCUSSION: Effective chronic treatment with FLUV appears to reduce YOH-induced anxiety and somatic symptoms. These results suggest that interactions between the 5-HT and noradrenergic systems may have a role in the therapeutic mechanism of the 5-HT reuptake inhibitors in PD.

I DBA neurons have cell bodies (A group) in the median zona incerta (MEI), in the anteroventral periventricular medial nucleus (DMN). The concentration of norepinephrine (NE) in these regions was assessed, reflecting a higher density of MEI nerve terminals; this can complicate interpretation of studies employing neurotochemical techniques. The purpose of this study was to determine if concentrations of the DA metabolite DOPAC reflect the activity of IBDA neurons. Administration of dopamine agonist (DA) increased DOPAC concentrations in the DMN. Injection of an NE neurotoxin (5-saquinine) into the ventral ME bundle, reduced NE concentrations in the DMN by 80% while DA and DOPAC concentrations were unaltered. Activation of ME neurons with the DA-agonist receptor antagonist idazoxan increased DOPAC concentrations of 3-methoxy-4-hydroxyphenylethylamine and DOPAC in MEI and DMN in intact but not in ventral ME bundle-ligated rats. These results reveal that DOPAC in MEI and DMN reflect the activity of IBDA neurons, but that when NE neurons are activated, significant amounts of DA are metabolized to DOPAC in MEI terminals in these regions. (Supported by NIH Grant NS 15911.)

CATECHOLAMINES VI

THURSDAY PM

1178

PREVIOUS EXPOSURE TO CHRONIC STRESS RESULTS IN ENHANCED NOREPINEPHRINE SYNTHESIS IN RESPONSE TO A NOVEL STRESSOR. L. A. Narins, M. E. Dean, S. E. Abood, Department of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

We have shown that chronic stress results in enhanced hippocampal norepinephrine (NE) release in response to a novel stressor (Dean and Narins, 1, 4, 1989). One factor that may contribute to this phenomenon is increased synthesis of neurotransmitter. To test this hypothesis, several measures of NE synthesis were measured using both chronic and chronically stressed rats. Analysis of hippocampal tyrosine hydroxylase (TH) activity in vivo revealed no significant difference between the two groups. In view of the latter finding, the chronic stress does produce a detectable increase in the maximal amount of hippocampal TH enzyme activity in vivo. The synthesis of NE in vivo was assessed in two ways. First, the accumulation of 3,4-dihydroxyphenylalanine (DOPA) in hippocampus 30 min after administration of the aromatic amino acid decarboxylase inhibitor NSD-I (100 mg/kg, i.p.) was assessed. Under basal conditions, DOPA accumulation was the same in both groups. In response to tail shock stress, however, the increase in DOPA efflux was larger in chronically stressed rats (130 ± 9%) than in naive animals (45 ± 8%). Second, the level of extracellular 3,4-dihydroxyphenylacetic acid (DOPAC) was quantified in the hippocampus using in vivo microdialysis. The basal level of DOPAC did not differ between the two groups. In response to tail shock stress, however, the increase in DOPAC accumulation in chronically stressed animals (101 ± 18%) was greater than in naive animals (55 ± 8%). While these findings may not be clearly interpretable, the results suggest that these two groups of animals, activation of TH by a novel stressor appears to be greater in chronically stressed rats than in controls.

D2 DOPAMINE AGONISTS ACUTELY INCREASE THE ACTIVITY OF TUBEROINFUNDIBULAR DOPAMINERGIC NEURONS. P. E. Moore, C. K. Goudreau, M. B. Goudreau, Departments of Pharmacology and Psychiatry, Case Western Reserve University, Cleveland, OH 44106.

The activity of tuberoinfundibular dopaminergic (TIDA) neurons, in contrast to that of nigrostriatal neurons, is not acutely affected by typical dopamine antagonists, e.g., haloperidol, or agonists. However, we have reported that the activity of tuberoinfundibular dopaminergic (TIDA) neurons is acutely suppressed by D2 dopamine agonists (Neurosci. Abstr., 15:1000, 1989). In the present study, we have examined the effects of the selective D2 agonist quinpirole and quinolinol on the activity of TIDA neurons. Both D2 agonists were applied from DOPAC concentrations and the in vivo accumulation of DOPA after decarboxylase inhibition in the median eminence. The accumulation of DOPA in the median eminence was dose-dependently increased by quinpirole (0.1-2.5 mg/kg, ip) and quinolinol (0.025 mg/kg, ip). Quinpirole and quinolinol also significantly increased the concentration of DOPAC in the median eminence. The activity of TIDA neurons was increased within 1 hr after the administration of quinpirole, and it remained elevated for 4 hrs. The stimulatory effect of quinpirole on TIDA neurons was completely antagonized by haloperidol (1 mg/kg, ip), but was unaffected by SCH 23990 (0.5 mg/kg, ip). It is concluded that D2 receptor stimulation results in an acute increase in the activity of TIDA neurons.


Dopamine-containing (DA) neurons in the arcuate nucleus of the guinea pig were recorded in hypothalamic slices. DA neurons, identified immunocytochemically by the presence of tyrosine hydroxylase, had a mean somata width profile of 14.9 ± 4.4 X 11.5 ± 3.1 µm (N = 14), a Na⁺ action potential of short duration (0.8 ms) and an after-hyperpolarization of 6.9 mv with a decay half-time of 53 ms. Following induction of repetitive firing (20 to 50 Hz), DA cells exhibited a low threshold spike (LTS) which induced a 140 mV action potential. The LTS was identified as an inward current which activated positive to -70 mv and was sensitive to high K⁺/low Ca⁺ media but not to TTX. DA neurons also had a large depolarizing inward current at potential between the 70 to -70 mv. Ca⁺ blocked this conductance. The µ-opioid agonist [D-furyl-D-Ala₂-Gly-NH₂]-enkephalin (93 ± 44 PA) which had a reversible potential similar to that of a selective norepinephrine conductance (NACH) did not block the opioid effects. Therefore, DA neurons of the arcuate nucleus differ in their intrinsic conductances and their responsiveness to μ-opioid and norepinephrine (NE) neurons. Furthermore, opioid activation of a potassium conductance in DA neurons of the arcuate nucleus may underlie the effects of opioids on dopamine-mediated prolactin release.

1179

CENTRAL D1 AND D2 DOPAMINE RECEPTORS STIMULATE HYPOPHALAMIC-PITUITARY-ADRENAL (HPA) ACTIVITY. B. Brozovsky and C. M. Kubi, Dep. of Pharmacology, Duke University Medical Center, Durham, NC 27707.

Non-selective and D2 selective DA agonists as well as cocaine and other inhibitors of DA uptake stimulate the secretion of ACTH and corticosterone (CS) in rats. We now report that both D1 and D2 DA receptors contribute to the control of the HPA axis. A large fraction of the dopaminergic input to the intermediate third ventricle, such as the hypothalamus, may be involved in this response. Both the D1 selective agonist (SKF38393) (1 to 20 mg/kg, ip) and the D2 selective agonist quinpirole (0.3 mg/kg, ip) produced dose-dependent elevations in serum ACTH and CS. The HPA response to SKF38393 was attenuated by the D1 selective antagonist SCH 23390 (0.2 mg/kg, ip) while the response to quinpirole was attenuated by the D2 selective antagonist (S)-3-pirole (50 mg/kg). Administration of either SKF38393 or quinpirole (1 to 100 mg/kg, ip) in the indwelling cannula caused a dose-dependent elevation in ACTH secretion. Pretreatment with SCH23390 or (S)-3-pirole (100 mg/kg, ip) attenuated the ACTH response to SKF38393. Similarly, administration of the selective DA uptake inhibitor GBR12909 (3 to 60 mg/kg) dose-dependently elevated serum ACTH and CS levels. These results establish the third ventricle as a role in the regulation of HPA activity through actions on both D1 and D2 receptors. In addition, the DA system mediating this endocrine response appears to be independent from forebrain DA systems mediating locomotor and rewarding responses. Supported by NASA PSO 20503-01A.
488.1 EFFECT OF LONG TERM TREATMENT WITH PHORBOL ESTERS ON THE RELEASE OF NORADRENALINE FROM THE MOUSE ATRIA. S. Foucart, J.-F. Morange, R. Molinewski and J.-L. Chevalag, Depart. of Pharmacology, University of Melbourne, Parkville, Victoria, 3052, Australia.

The role of protein kinase C (PKC) in sympathetic neurotransmission was investigated by down regulation induced by long term exposure to phorbol esters. In the present study, mouse atria were incubated for 10 hours at 37°C in the presence of either phorbol myristate acetate (PMA, 1 μM) or phorbol dibutyrate (PDB, 1 μM). The atria were then incubated with [3H]-noradrenaline for 60 min followed by 60 min of washing, the atria were field stimulated at 5 Hz and the stimulation-induced (S-i) outflow of radioactivity was taken as an index of noradrenaline release. In control experiments, PMA (1 μM), PDB (1 μM) or a combination of 8-bromo cyclic AMP (90 μM, 8-Br-cAMP) and dibutyryltetraethylenelimine (100 μM, IBMX) significantly increased the S-i outflow to 177%, 230% and 169% of control respectively. Pretreatment with PMA or PDB did not alter the absolute release of noradrenaline, it appears that protein kinase C is not implicated in the noradrenaline release process.

488.3 ROLE OF PROTEIN KINASE C (PKC) IN MEDIATION OF DYNORPHIN-(1-8) (DYN) AND MET*-ENKEPHALIN (MET) RELEASE ASCREASED BY ANGIOTENSIN II (ANG), ARACHIDONIC ACID (AA) AND PGE₂ IN PRIMARY CULTURED BOVINE CHROMAFFIN CELLS. H. H. Sun, M. K. McMillan, P. Hudson* and J. Hong. DNIV, NIH/NIH, Research Triangle Park, NC 27709.

We have previously reported that 24 hr treatment of chromaffin cells with ANG, AA and PGE₂ caused an increase of MET secretion. In the present study, effects of ANG, AA and PGE₂ on the release of DYN were examined in bovine chromaffin cells. MET and DYN contents were measured by radioimmunoassay. We found that long-term stimulation (24 hrs) of the chromaffin cells with ANG (20 nM), AA (100 μM) and PGE₂ (10 μM) caused increased in secretion of DYN as well as MET. The amount of DYN secreted by cells was 100-fold less than MET. To examine possible involvement of PKC, effects of protein kinase inhibitors on increases in secretion of MET and DYN induced by ANG, AA and PGE₂ were studied. Staurosporin (10 μM), a protein kinase inhibitor which has a high affinity (Ki=0.7 μM) for PKC, almost completely inhibited increased release of MET and DYN induced by ANG, AA and PGE₂. However, an equal nanomolar concentration of K252a, a protein kinase inhibitor which has a low affinity (K=25 μM) for PKC, had no effect. Our results indicate that PKC appears to be involved in increased secretion of MET and DYN induced by ANG, AA and PGE₂.

488.6 ABSENCE OF A CHANGE IN DARPP-32 PROTEIN OR mRNA IN NEONATAL-6-OHDA-LESIONED RATS. G.R. Breese, J.O. Callaghan, N. Ebrol, H.R. Cruvellier, R.A. Mueller and P. Greenberg, UBC School of Medicine, Chapel Hill, NC; EPA, RTP, NC; and Rockefeller University, NY.

DARPP-32 is a protein that is enriched in neurons associated with DA₁-receptors. Neonatal-6-OHDA (N-6-OH) lesioned rats are known to develop DA₁-receptors after repeated treatment (i.e., primed) with a DA₁ agonist. The present investigations determine whether priming of this response in N-6-OH lesioned rats is modified by either focused microwave irradiation or decapitation, respectively, for these determinations. DARPP-32 content in the striatum, measured by immunobassay, was not affected by any of the treatments when compared to the concentration in unlesioned rats. A change in DARPP-32 mRNA as measured by Northern blot analysis was also not apparent in N-6-OH lesioned rats after priming of DA₁-receptor responses. Studies are underway to determine if the content of phosphorylated DARPP-32 is altered in N-6-OH lesioned rats.
488.7


We are developing a cell culture assay for studying the short- and long-term effects of dopamine on protein phosphorylation and gene expression in the nervous system. We chose DARPP-32 as a model protein because its phosphorylation is increased by dopamine in vivo, and because the rat and mouse DARPP-32, pM, available. We tested PC12 (rat adrenal pheochromocytoma) cells first because of their similarity to neuronal cells, and because DARPP-32 is found in rat brain, but not in human brain. PC12 cells were incubated with dopamine in RPMI medium supplemented with ascorbate. The cells were collected, rapidly frozen and homogenized on ice. Half of each sample was incubated for 30' at 37°C to allow endogenous phosphatases to remove all phosphate groups, and thereby permit measurement of total phosphorylatable sites. Both sample halves were then quenched with zinc acetate, dissolved in citric acid, neutralized, phosphorylated by exogenous cAMP-dependent protein kinase and [γ-32P]-ATP, resolved by SDS/PAGE and autoradiographed. A band at M, 32K was phosphorylated in a dose and time dependent manner. The phosphorylation of numerous other bands was not affected. We are currently attempting to confirm that the band is DARPP-32, and to determine whether the effect is D1, D2 or autoreceptor specific.

488.8

POTENTIAL OF ENDOTHELIN- AND ATP-INDUCED PHOSPHOinosi- TIDE TURNOVER BY CALCIUM IONOPHORES IN C6-GLIA CELLS. M.-H. Liu, C.-Y. Lee, and D.-H. Chang. Biological Psychiatry Branch, NIMH, Bethesda, MD 20892 and Dept. of Pharmacology, National Taiwan University, Taipei, Taiwan

Endothelin-1 (ET) and ATP induce a robust increase of phosphoinositide (PI) metabolism in C6 glioma cells and primary cultures of cerebellar astrocytes. In this study, we investigated interaction between Ca²⁺ (Lumophore, A23187) and ETF and ATP-induced inositol phosphate [3H-IP₃] in these cells prelabeled with 3H-myoinositol. In C6 glioma, A23187 dose-dependently potentiated the responses to ET and ATP with a dose of about 0.1 µM. The maximal stimulation of PI turnover induced by ET (30 nM) and ATP (100 µM) was increased from 2.4 to 42-fold and from 8 to 19-fold, respectively, by the presence of 10 µM A23187, which alone induced a 3.5-fold increase of [3H-IP₃] accumulation. The EC₅₀ values of ET (2 nM) and ATP (84 µM) were unchanged by this Ca²⁺ (Lumophore). Inosine also potentiated the efficacy of ET-induced PI hydrolysis in concentration range of 10⁻⁴-10⁻⁶ M. In contrast to Ca²⁺-glioma, cerebellar astrocytes A23187 failed to induce synergistic effects on PI metabolism induced by ET, ATP, norepinephrine, angiotensin, bradykinin, and neurotransmin, although the angiotensin alone induced a more than 4-fold increase in [3H-IP₃] accumulation. These results indicate that entry of Ca²⁺ has an important role in amplifying PI turnover stimulated by ET and ATP in C₆-gloma cells but not in cerebellar astrocytes.

488.10


The bag cell neurons (BCNs) provide a model system for the study of neuromodulator release and its regulation by second messengers. Following electrical stimulation, the BCNs fire repetitively (afterdischarge) and release several neuropeptides including egg-laying hormone (ELH). During the afterdischarge, CAMP levels increase and phosphoinositide (PI) hydrolysis is stimulated leading to an activation of the kinases PKA and PKC. We have tested the effects of H8 and H7, two kinase inhibitors that act in intact cells with preferential specificity for PKA and PKC respectively, on the release of ELH. BCNs in intact ganglia were arterially perfused with artificial seawater (ASW), H8 (100µM) or H7 (30-100µM) for 30 min prior to stimulation of an afterdischarge. The surrounding medium was exchanged at 5 min intervals throughout the afterdischarge and the released material analyzed using HPLC. Release at each 5 min interval was monitored as total ELH release during the entire afterdischarge. Afterdischarges were reduced in both H8 (n=6) and H7 (n=10) compared to ASW controls (n=8). These results were found with afterdischarges lasting over a wide range of durations (range: 10-35 min). In sum, these data suggest that both PKA and PKC may modulate the amount of ELH released during an afterdischarge.

488.11

CORRELATION OF PHORBOL ESTER-INHIBITED PHOSPHOHISTIDINE METABOLISM WITH PROTEIN KINASE C-MEDITATED PHOSPHORYLYATION OF RAT HIPPOCAMAL MEMBRANE PROTEINS. L. M. Stuffer, Y. F. Han, and A. S. Dokchitser. Departments of Biochemistry and Neurology, Medical College of Ohio, Toledo, OH 43699.

In the brain, acetylcholine stimulates the breakdown of phosphorylhistiso- toid 4.5 bisphosphate (PIP₂) to inositol triphosphate and diacylglycerol (DG), which activates protein kinase C (PKC). Active phorbol esters, which have been shown to block cholinergic stimulation of PIP₂ breakdown and which stimulate PKC, are known to stimulate membrane-associated protein substrates of this enzyme. When rat hippocampal slices were incubated with 10 µM 12-O-tetradecanoyl-phorbol-13-acetate (TPA), there was inhibition of cholinergic stimulation of PIP₂, which was associated with altered phosphory- lation of two membrane proteins. Using 2-dimensional gel electorephore- sis, post-hoc phosphorylation assays and western blotting, one protein was identified as a component of a major protein component with a P₁ of about 69, increased phosphorylation of this protein is positively modulated by inclusion of hippocampal slices with TPA. Further characteristic of this protein is currently underway. Supported by grants from NIH (NS 32592) and the Ohio Department of Aging.

488.12


Protein phosphorylation is recognized as a primary regulator of cell metabolism, including stimulated-secretion of hormones. In the past we have demonstrated the presence of cAMPA, calcium/potassium- and calcium-calmodulin-stimulated protein phosphorylation of endogenous anterior pituitary protein substrates in crude homogenates of the rat anterior pituitary. We have extended these findings to investigate the presence of endogenous anterior pituitary protein phosphorylation in crude supracellular fractions of the rat anterior pituitary.

Normal male rats were killed by decapitation and the anterior pituitary were removed and homogenized in ice cold buffer. The homogenates were centrifuged at 100,000g x 1 h, and the supernatant and digested the cytosolic fraction. The pellet was resuspended in the same volume of buffer and digested the particulate fraction of the tissue specimen. Protein phosphorylation was performed in the presence and absence of calmodulin (5 µg/ml), the calcium calmodulin kinase II inhibitor mastoparan (3 µM), phospholipid serine (50 µg/ml), and calcium (1mM). The reaction was carried out for 60 seconds. The samples were boiled and electrophoresed on 10% acrylamide SDS-PAGE gels. Autoradiographs of the gels were prepared and analyzed by scanning densitometry.

Proteins with molecular weights of 85.5 and 53.2 kD were identified in the cytosolic fraction to be phosphorylated in the presence of calmodulin (putative calcium/calmodulin kinase II activity), and those with molecular weights of 74.3 and 17.4 kD were identified in the cytosolic fraction to the phosphorylated for protein kinase C. Those with molecular weights of 23.3 kD, 19.7 kD, and 14.1 kD were identified in the particulate fraction to be phosphorylated by calcium/calmodulin kinase II, and those of 92.1 & 99.0 kD were identified as the phosphorylated protein kinase C.

(Supported by USPHS grants MH18921 & MH 29228)
488.3 PROTEIN KINASE C ISOFORMS DISTINGUISH MAJOR CELL TYPES IN RAT HIPPOCAMPUS. J.F. McGinity, W.T. Bohle*, M. Coupe*, and W. West*, Dept of Anatomy and Cell Biology and Medicine, East Carolina University School of Medicine, Greenville, NC 27858.

Protein kinase C (PKC) types I, IIa (alpha/beta), and IIb (epsilon) immunoreactivity (IR) for PKC have been reported to be present in putative hilar basket cells and pyramidal cells of the hippocampal formation (Stitcher and Singer. Exp. Brain Res. 72:149. 1988). The present study investigated the distribution of PKC alpha, beta, and epsilon immunoreactivity (IR) and mRNA in the hippocampus using selective antisera and oligonucleotide probes in immunocytochemistry (ICC) and in situ hybridization histochemistry (ISHH) respectively. PKC alpha antisera was raised against synthetic peptides with non-overlapping sequences and were found to be specific for each isoform by Western blot analysis. ICC was performed with standard avidin-biotin-peroxidase method. PKC alpha antisera preferentially stained two sets of neurons: hilar neurons aligned in a basket cell subfield subjacent to the granule cell layer displayed homogeneous cytoplasmic IR whereas CA3 pyramidal cells displayed a punctate reaction product (Stitcher and Singer's type 1 and 2 cells). The neuropil in stratum radiatum and stratum oriens was also labeled in the terminal distribution pattern of Schaffer collaterals. PKC beta antisera stained CA3 pyramidal cells most intensely with a punctate distribution of IR. PKC epsilon antisera stained dense granule cells and mossy fibers most intensely. Absorption controls demonstrated the specificity of each antisera. In situ hybridization revealed that mRNA substantiated the distributions revealed by ICC; PKC alpha mRNA was denser in CA1 pyramidal cell layer although the CA1 pyramidal cell layer contained a less intense signal. The opposite distribution was true for PKC beta mRNA whereas PKC epsilon was most intense in the granule cell layer. Thus, different patterns are preferentially expressed by the major cell types in the hippocampal formation. The regulation of expression of these different isoforms is under investigation. Supported by DA 03982.


Protein phosphatase 2A (pp2A), identified in rat hippocampus as a histone protein phosphatase activity, was totally inhibited by 1 mM okadaic acid. Substrate specificity studies suggested that pp2A regulates autophosphorylated Ca2+ and AMP-dependent protein kinases. With neuronal depolarization, pp2A activity redistributed from cytosol to synaptic plasma membranes. Purified rat brain pp2A is a trimmer of 63, 25 and 38 kDa polypeptides. Amino acid (AA) analysis of the 63 and 38 kDa subunits revealed a composition nearly identical to the 65 and 38 kDa subunits, respectively, of pig kidney and rabbit skeletal muscle pp2A. Sequencing & AA analysis of 2 of 6 isolated CNBr fragments of the 38 kDa subunit suggest it is highly homologous to published sequences of the pp2A catalytic subunit. Sequencing & analysis of a 17 kDa CNBr fragment & a 12 kDa trypsin fragment of the neuronal 63 kDa subunit suggest its primary structure is highly homologous to the isoform of the 65 kDa subunit from pig kidney and HeLa cells. Supported by V.A. Medical Research Funds.

EXCITATORY AMINO ACIDS: NON-NMDA RECEPTORS


D,L-2-amino-3-phosphonopropionic acid (AP3), a phosphonate-substituted derivative of aspartic acid, has been shown to be an inhibitor of excitatory amino acid-stimulated phosphoinositide hydrolysis in rat brain slices. In this study, the enantiomers of AP3 were synthesized and used to further characterize the stereoselectivity and mechanism of interaction of this compound for inhibiting phosphoinositide-coupled (metabotropic) excitatory amino acid receptors. L-AP3 was 3.5 times more potent than D-AP3 as an inhibitor of inositol-stimulated 32P-insoluble phosphatase formation in slices of the rat hippocampus or quisqualate-stimulated 32P-insoluble phosphatase formation in neonatal rat cerebral cortical slices. Carbamyl-stimulated phosphoinositide hydrolysis was not inhibited by L-AP3, and L-AP3 had no appreciable activity for ionotropic excitatory amino acid receptors at concentrations required to inhibit metabotropic excitatory amino acid responses. The inhibitory effects of L-AP3 or D-2-amino-4-phosphonobutyric acid on phosphoinositide hydrolysis were non-competitive since they could not be surmounted by increasing concentrations of ionophore or quisqualate. L-AP3 inhibition also could not be prevented by washing the tissue prior to incubating with ionophore. Although L-AP3 is a stereoselective inhibitor of metabotropic excitatory amino acid receptors with little affinity for ionotropic receptors, the site at which it acts to inhibit metabotropic excitatory amino acid receptors remains to be determined.

468.2 PHARMACOLOGICAL BLOCKADE OF OSCILLATORY CURRENTS INDUCED BY TRANS-ACPD AND QUISQUATE IN RAT BRAIN mRNA-Injected XENOPUS OCYCTES. G.D. Watson, D.T. Monaghan* and T.H. Lanham. CNS Diseases Research, G.D. Searle & Co., Skokie, IL 60077 and Dept. of Pharmacology, University of Nebraska, Omaha NE 68105.

It has been suggested that trans-ACPD may be a selective agonist for the metabotropic quisqualate-sensitive receptor. In X. oocytes injected with rat cerebral cortical RNA, quisqualate induced both smooth and oscillatory currents indicating that it acts at more than one receptor. Trans-ACPD induced only oscillatory currents suggesting that it selectively activates the metabotropic receptor. The threshold concentration for trans-ACPD was approximately 30 µM. Oscillatory currents induced by both quisqualate and trans-ACPD were blocked by intracellular injection of EGTA. These oscillatory currents were also blocked by the application of 30 nM NMCA plus 10 µM glycine but not by NMDA alone. Depolarizing current injection did not mimic the effect of NMDA plus glycine. Oscillatory currents which had been blocked by EGTA could occur when NMDA plus glycine was co-applied with trans-ACPD. Smooth currents induced by quisqualate were unaffected by either EGTA or NMDA plus glycine. Oscillatory currents were not blocked by the non-selective acidic amino acid antagonist DACH (10 µM).
489.3

—(Carboxycyclopentyl) glycine (CCG) is a conformationally restricted analog of glutamate and has eight stereoisomers theoretically. The (S,S,4S) isomer of CCG (L-CCG-1) caused a marked depolarization due to activation of non-NMDA, non-kaolinate AMPA type receptors. The development of this new and potent agonist opens the possibility for extensive studies of the glutamate receptor subtypes. Peak amplitudes of responses to a possible metabotropic agonist, trans-ACPD, and L-CCG-1 were significantly decreased when the temperature was decreased, while those of other excitatory amino acids was markedly increased, suggesting that the intracellular enzyme systems were related to the depolarization induced by L-CCG-1. Application of L-CCG-1 induced the early responses in the Xenopus oocyte with injection of mRNA from rat brain, suggesting that L-CCG-1 is a metabotropic receptor agonist. L-CCG-1 is more potent than trans-ACPD in causing a depolarization in the rat isolated spinal cord. L-CCG-1 depressed monosynaptic reflexes of the rat isolated spinal cord in considerable concentrations. At the moment, it is not clear that this depression of monosynaptic reflexes is through activation of metabotropic receptors.

489.4
SENSITIZATION TO L-AMINO-4-PHOSPHOBUTANOIC ACID (L-AP4) IS INDUCED BY AGONISTS SELECTIVE FOR PHOSPHOINOSITIDE-LINKED RECEPTORS. E.A. Whittemore and C.W. Cotman. Dept. of Psychology, Univ. of Calif., Irvine, CA 92717

Quisqualic acid (QA) sensitizes neurons to depolarization by D- and L-2-amino-4-phosphobutanoic acid (AP4). This 30- to 100-fold sensitization persists for hours through an interaction of QA with a receptor distinct from the QA/AMPA receptor ionophore. Only QA has been shown to induce this 'QUIS-effect'. In order to test whether the QUIS-effect is induced via the (S)-trans-2-amino-4-phosphono-5-carboxylate (S)-trans-2-amino-4-phosphonobutanoic acid and carbamyl (300 μM). These compounds and concentrations are reported to stimulate phosphoinositide (PI) turnover in biochemical assays. However, the sensitization induced by these compounds was less (2- to 5-fold) than by QA (30- to 50-fold), and reversed much more rapidly. For example, a 4-minute exposure to 100 μM trans-ACPD shifts the IC50 for L-AP4 from the pre-exposure value of 1800 μM to approximately 500 μM. In contrast, 16 μM QA shifts the IC50 for L-AP4 to 55 μM. These data suggest that the QUIS-effect may be induced in part via a PI-linked mechanism and may represent a long-term physiological response to PI turnover in the CNS.

489.5
Effects of Quisqualate on Cytosolic Free Calcium in Single Cultured Rat Cerebellar Granule Cells. A.J. Irvine* G.L. Collingridge* F. S. Bannister* A.J. Sheardown* (SFON: Brain Research Association) Departments of Biochemistry, Pharmacology and Zoology, University of Bristol, Bristol, BS8 1TD, UK.

Quisqualate can mobilize intracellular free calcium ([Ca2+]i) via a receptor that is linked to phospholipase C. This 'metabotropic' receptor is believed to be present on cerebellar granule cells. We have investigated the relationship between activation of this receptor and calcium mobilization, obtaining simultaneous measurements from several individual granule cells within a field. Cells were loaded with FURA-2/AM and imaged using an intensified CCD camera linked to a Joyce-Loebel magical system.

In Ca free medium, 1 μM quisqualate caused a rapid transient elevation of [Ca2+]i. This effect did not occur with repeated applications but could be recovered following exposure to 1 mM Ca2+. Reproducible transient responses could be obtained by applications of 1 μM quisqualate in the presence of 1 mM Ca2+ and 20-40 μM CNQX. In some cells, these responses were reversibly antagonised by 1 μM DL-AP4. These results are consistent with the notion that granule cells contain a Ca mobilising, quisqualate-activated receptor.

489.6

2,3-dihydroxy-6-nitro-7-sulamoyl-benzo(f)quinazoline (NBQX) is a potent and selective inhibitor of binding to the ionotrophic quisqualate receptor. IC50 for inhibition of 1H-AMPA binding = 150 μM. NBQX protects against global ischemia when administered up to 6 hours after reperfusion. The present report examines the effect of NBQX on the quisqualate receptor, coupled to inositol phosphohydrols (IPH). The effect of excitatory amino acids on IPH (measured by the accumulation of inositol monophosphate in the presence of LiCl) in cerebellar granule cells in culture from 7-day-old mice was examined. Quisqualate (0.1-300 μM), kainate (10-300 μM) and glutamate (10-300 μM) stimulated IPH in a dose-dependent manner. The ability of quisqualate to stimulate IPH was unaffected by CNQX (50 μM), whereas APV (10 μM) blocked the increase in inositol phosphohydrolsysis produced by glutamate. NBQX (0.1-300 μM) did not alter the stimulation of IPH produced by quisqualate (100 μM). NBQX (30 μM) also had no effect on the ED50, value for quisqualate stimulated IPH. The effect of NBQX in inositol and kainic acid stimulated IPH will also be discussed. These data suggest that the neuroprotectant effect of NBQX is mediated via the ionotropic, but not the metabotropic, quisqualate receptor.

489.7
CNQX and APV INSSENSITIVE GLUTAMATE RESPONSES IN RAT SUBSTANTIA GELATOSINea NEURONS. M. Yoshitma & T. M. Jessell. Center for Neurobiology and Howard Hughes Medical Institute, Columbia University, New York, NY. 10025

We have examined the pharmacological properties of l-glutamate evoked responses and primary afferent-evoked epsps in substantia gelatinosa (s.g) neurons using the RP-2830, non-NMDA, non-kaolinate AMPA receptor antagonist CNQX (5 μM). The CNQX-resistant component of the glutamate-evoked response did not result from activation of NMDA receptor.

We also examined glutamate-evoked currents in neurons isolated from the superficial dorsal horn of adult rat spinal cord. In about 60% of neurons, inward currents evoked by glutamate, quisqualate (QA) and kainate (KA) were almost completely eliminated by CNQX (10 μM). However, in 40% of neurons, CNQX depressed glutamate responses by only about 30%, even though QA and KA responses in the same neuron were depressed by more than 90%. The CNQX-resistant glutamate response was not blocked by APV. These observations suggest that glutamate-evoked currents in this population of neurons are mediated by non-NMDA glutamate receptors with different sensitivities to CNQX.

489.8
NBQX, (2S,3R)-DIHYDROXY-6-NITRO-7-SULFAMOYL-BENZO(f)QUINOXALINE: ANTICONVULSANT EFFECTS IN MICE. M. D. B. Sydmarken, P. Jacobsen & T. Honore*. Novo Nordisk, Perronas CNS Division, Sydmarken 5, DK-2860 Soeborg, Denmark.

NBQX, an analog of the quinoxalinedicarboxylic acid antagonists to non-NMDA glutamate receptors, potently and selectively inhibits AMPA receptor binding with no activity at NMDA or glycine sites, and protects from cortical ischemia after global ischemia (1). The anticonvulsant effects of NBQX were evaluated by the intraperitoneal (ip) and the intracerebral (iv) route against audiogenic seizures in DBA/2 mice, and against seizures induced by 150 mg/kg DMCM (ip: 30 min) in NMRI mice, at 15 and 30 min prior to test. Sedation was assessed in a rotorod apparatus.

In DBA/2 mice NBQX was 5-17 (ip) and 2-3 (iv) times more potent than an anticonvulsant, ED50's (mg/kg) being 13.0, 8.2 and 3.5 (ip) and 15.5 and 15.5 (iv) at 5, 15 and 30 min, respectively, than in producing sedation. NBQX was ineffective at doses up to 30 mg/kg (ip), or ataxic at doses equal to, or lower, than the anticonvulsant doses (iv).

NBQX shows anticonvulsant effects in mice probably by suppressing the activity of the non-NMDA excitatory amino acid quisqualate.

489.9

QUISQUALATE RECEPTOR ANTAGONISTS IN THE SUB-PALLIUM (SP)
DECREASE THE HYPERMOTILITY RESPONSE TO AMPHETAMINE. D.L.
WILLIAMS*, D.S. SUPPE*, R.A. HILL†, L.J. WALLACE*, D.C.
MILLER* and N.J. URETZKY†. College of Pharmacy, The Ohio
State University, Columbus, OH 43210.

Previous studies suggest that quisqualate (QUIS) receptors
are involved in the stimulatory aspect of locomotion (LMA).
AMPA, a QUIS agonist, upon injection into the SP, stimulates LMA. This effect
is antagonized by the non-NMDA receptor antagonist CNQX. We
have recently found that both CNQX and DNQX can inhibit
the hypermotility response to systemically administered
amphetamine (AMPH). In order to determine whether the
inhibitory effects of DNQX are meditated through the
blockade of QUIS receptors, we have evaluated the effects
of structural analogs of DNQX on AMPH binding and on
their ability to inhibit the hypermotility response to
AMPH. DNQX, 2-hydroxy-3,5-dinitrotyrosine (DNT), and
AFQX, an analog of DNQX, inhibited the binding of
AMPH with IC50 values of approximately 0.5 μM, 10 μM, and
100 μM respectively. The doses of DNQX and DNT that inhibited
AMPH-stimulated locomotion by 50% were 0.4 and 3.4 nmoles
respectively, while AFQX at a dose of 5 nmoles did not
exert an inhibitory effect. The order of potency for these
compounds was similar for both inhibition of AMPH
binding and AMPH stimulated LMA. These studies suggest
that the ability of DNQX to inhibit the hypermotility
induced by AMPH is due to the blockade of QUIS receptors.

489.10

IN SITU HYBRIDIZATION OF OLIGONUCLEOTIDE PROBES TO mRNA ENCODING
A KAINATE SENSITIVE GLUTAMATE RECEPTOR (GluR-K). D.W. BOHANUS,
D.A. HOFSTED, Z. CAO* and J.O. MCMANUS. V.A. and Duke

The recent isolation of cDNA clones encoding functional excitatory amino acid
receptors (EAA) opens new avenues for investigating EAA receptor pharmacology.
One key step in characterizing cloned receptors is to compare the amino acid
sequence of the cloned molecule with pharmacologically and electrophysiologically
characterized endogenous receptors. Thus we used oligonucleotide probes to
map the amino acid sequence of an mRNA which encodes a kainate
activated, glutamate receptor. Two [32P] labeled probes, complementary to
overlapping regions of the Glur-KI clone (Hollman et al.), were hybridized to
10 μg sections of rat brain. Northern blot analysis showed that the probe bound
to a similarly sized band of poly A+ RNA. Similarly, the amino acid
sequence of the Glur-KI clone was identical for the two probes. The greatest density
of binding was in the hippocampus, in hippocampus, binding was enriched in the
dentate granule and CA3-1 pyramidal cell layers. The absence of a specific enrichment
of binding over the granule or CA3 pyramidal cells argues against this mRNA
encoding the high affinity [3H]kainate binding site localized to area
stratum lucidum. This suggests that the protein encoded by the Glur-KI clone,
while capable of generating kainate evoked currents, is not a part of the high
affinity [3H]kainate binding site. This in turn suggests that kainate evoked
currents are mediated by a receptor other than that defined by high affinity
[3H]kainate binding. Which (if any) of the pharmacologically characterized EAA
receptors includes the probe encoded by the Glur-KI clone is unknown.
However, the amino acid sequence of the mRNA together with the
electrophysiologic properties of the expressed Glur-KI clone raise the possibility
that this protein may be part of, a quisqualate insensitive, AMPA receptor.

489.11

ORNITHIC KAINATE TREATMENT DECREASES K+ STIMULATED RELEASE OF
ENDOGENOUS AMINO ACIDS FROM CULTURED CEREBRAL NEURONS. M.L.
Simmons and D.R. Dutton, Department of Pharmacology, University
of Iowa, College of Medicine, Iowa City, IA 52242.

Cultured rat cerebral neurons containing 20% granule
cells, 5-10% inhibitory interneurons and 3-5% glial cells were
used to identify the neuronal subclass of origin of released
endogenous amino acids. Cultures were treated for 4 days, 4-8
days in vitro (DIV), with 50 μM kainic acid (KA) which has pre-
viously been shown to selectively kill the GABAergic neuronal
population (Drejer and Schwabsehe Neurochem. Res. 14:751-756,
1989). These conditions, under which a 20% loss of glutamic
acid decarboxylase (GAD) immunoreactivity was seen, produced
a complete loss of KA-induced release of GABA, and a
significant reduction in the release of aspartate, glutamate,
taurine and adenosine at 9 DIV. These KA-induced effects were
prevented by concurrent treatment with 50 μM or not 10 μM,
6-cyano-7-nitroquinazoin-2,3-dione (CNQX). Treatment
with CNQX alone caused the release of all these substances above
control levels.

Results indicate that glutamate and aspartate originate from
granule neurons, GABA from inhibitory neurons, and adenosine
and taurine possibly from both neuronal classes. KA treatment
does decrease endogenous amino acid release from granule neurons by
possibly depleting releasable pools or by downregulating KA
receptors.

Supported by NS3632 and the Life and Health Insurance Medi-
cal Research Fund.

489.12

TOPA OXIDIZED IN SOLUTION TO FORM AN AMINO ACID WHICH IS
A NON-NMDA AGONIST. D.S. CRADDOCK, E. ALBERGIANO, R.H.
FORBES, and D.A. ROSENBURG. Dep. Physiol., U. of Pitts-
burgh Sch. Med., Pittsburgh, PA; Dep. Pharm., Northeast-
and Univ. Med. Sch., Boston, MA 02115.

Application of solutions of 2,4,5-trichlorophenethyl-
alanine (topa) to rat cortical neurons or to the chick
eyeup preparation produces membrane responses which can be
blocked by the non-NMDA antagonist CNQX (Soc Neuro-
Pharmacological activity depends upon the forma-
tion of an oxidation product. In order to identify the active
compounds, we have performed qualitative amino
acid analysis with ninhydrin detection on solutions of
dopa and topa, using a Beckman 7300 analyzer. Oxidized
topas was prepared by dissolving topa in 50 m sodium
phosphate buffer at pH 6.8. Two peaks were found, one at
the elution time for topa (20.7 minutes), containing 18% of
the starting material, and a new peak at 3.3 min-
utes, containing 51% of the starting material. Incubation
of the sample prior to analysis led to dissolution of this peak
without appearance of new peaks. These data suggest
that topas in solution oxidizes to a more
stable amino acid, which is likely to be topa guinone;
this derivative subsequently degrades to a substance
which is not an amino acid. Of note, topa has recently
been demonstrated in bovine plasma amino oxidase.

489.13

LY207328 AND LY207193: TWO POTENT KAINIC ACID AGONISTS.
and D. L. DAVEY. Lilly Research Laboratories, Eli Lilly and Company,
Indianapolis, Indiana 46285 and Royal Veterinary College, London,"NR107U, UK.

Kainic acid (R1=CH3, R2=CH2, R3-H), isolated from Dipena
simplex, is a powerful excitatory amino acid (EAA) agonist and
neurotoxin. We wish to report two new potent kainic acid (KA)
agonists, LY207328 (R1=Ph, R2=CH2, R3-H) and LY207193
(R1=CH3, R2=NOH, R3-H). LY207328 and LY207193 displace
[3H]KA with IC50s of 0.40±0.1 nM and 36.5±0.8 nM, respectively.
In addition, LY207193 (R1=CH3, R2=OH, R3=OH) demonstrated
a more modest affinity for the 3H-KA binding site with an IC50 of
526 nM. Both LY207328 and LY207193 were found to stimulate
the release of [3H]norepinephrine from rat hippocampal slices as
cells as the release from rat retinal tissue.

LY207192 produced only a minimal response in the release
assays. In electrophysiological studies on rat cortical wedges, LY207328
and LY207193 were potent excitatory agonists. These responses
were sensitive to CNQX but not to
GS519755, suggesting an action via non-
NMDA receptors as it might be predicted from their
structural similarity to KA.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
**490.1**

**NMDA-EVOKED OUTWARD CURRENTS IN CULTURED NEOCORtical NEURONS USING NYSINT-PERFORATED PATCH RECORDINGS.** J. H. Habert and D. M. Kintner.

NMDA receptors were used to examine whole-cell responses to NMDA under conditions where loss of cytoplasmic factors was minimized and endogenous calcium buffering mechanisms were operational. NMDA was contained in 10 mM HEPES and 100 μM/mg NMDA. At -80 mV, responses to 100 μM NMDA were biphasic, consisting of an initial inward current which decayed to zero within 20 ms, and a slower outward current. The rundown of either component was seen. Steady-state peak ratios were similar in nystatin and conventional recordings suggesting similar rates of desensitization. When holding potentials from -40 to 0 mV were used, the initial inward current was followed by a slowly developing outward current. Near 10 mV, NMDA evoked an initial small decaying outward current followed by a large sustained outward current. At more positive holding potentials, NMDA evoked an initial outward current which decayed to a steady-state value. If cesium was substituted in the pipet, NMDA-induced outward currents were not observed.

Thus, under conditions where endogenous calcium buffering mechanisms are operational, NMDA evoked outward currents in cultured neocortical neurons. This is attributed to activation of a Ca-dependent K current via Ca entry through NMDA channels. (Supported by NS11845)

**490.2**

**WHOLE CELL PATCH-CLAMP RECORDING OF ENDOGENOUS SYNAPTIC CURRENTS IN MAMMALIAN MOTORONIONS IN INTACT BRAINSTEM-SLICE.** J. L. Kepes and J. W. Berger. System Neurobiology Laboratory, Dept of Kinetology, UCLA, Los Angeles, CA 90024-1568.

We previously reported that an excitatory amino acid (EAA)-like substance is the neurotransmitter involved in the bulbo-spinal transmission of inspiratory drive to phrenic motoneurons (Liu & Feldman, Soc. Neuro. Abstr. 15:4543; 1989; Liu et al. J. Neurophysiol. In Press). To further elucidate this EAA-like substance, whole cell patch-clamp techniques were employed in a brainstinal-spinal cord preparation in vitro (kshd). We used standard intracellular recordings to locate the phrenic motoneuron pool, then lowered a patch electrode (-3.5 MΩ) into the pool (-180-260 μm from surface). The tip of the patch electrode was kept clean by continuous positive pressure. Gigaspaces seals (-1.5 GΩ), and whole cell and constant current obtained by suction. The measured input resistances of these motoneurons were 100-500 MΩ, compared to values of -20-50 MΩ from standard intracellular recordings. The electrode was judged to be attached to the soma if large whole cell capacitance, and appropriate shape and voltage-dependence of the fast Na current, were measured. Two types of synaptic current were found. One occurred during inspiration and is mediated by an EAA-like transmitter. The associated EPSCs had very fast rise-time (<0.2 ms) and decay-time (-3.5 ms) constants, reversal potentials near 0 mV, and were blocked by the non-NMDA receptor antagonist CNQX. The shape of change of EPSCs at different holding potentials suggest they are voltage-dependent. Another synaptic current was present during expiration. This current had slower rise-time and longer decay-time (40-90 ms) constants and reversed near -40 mV. We interpret the later current as an inhibitory synaptic current carried by GABA channels.

In summary, whole cell patch-clamp technique is feasible in neurons receiving endogenous synaptic drive. This should permit the study of synaptic transmission under more physiological conditions than in preparations that require stimulated release of neurotransmitter. Supported by NIH Grant NS 24742.

**490.3**

**QUINOLIDINE ACID EFFECTS ON HIPPOCAMPAL PYRAMIDAL NEURONS.** J. C. Neick, D. R. Grunau, and G. J. Halperin. Departments of Neurology and Neurobiology, SUNY, Stony Brook, NY 11794.

We examined effects of quinolinic acid (QA) on pyramidal neurons in the in vitro rat hippocampal slice. At concentrations ≥ 100 μM, this NMDA agonist was reported to decrease the amplitude of antidromically evoked action potentials (APs) recorded extracellularly in this preparation (batch application (Stone)), and to depolarize cultured hippocampal neurons (pressure ejection from micropipette [Tuszuki et al.]).

Our extracellular recordings in the hippocampal stratum radiatum found that bath application of 100-200 μM QA depressed or abolished the EPSP and population spike evoked by stimulating the Schaffer collateral fibers (30% to 70%). Intracellular recordings disclosed that QA reversibly depolarized CA1 pyramidal neurons to a variable degree, and attenuated or eliminated EPSCs. Injecting depolarizing currents triggered slow, regenerative depolarizing waves. In some cells, QA eliminated APs evoked synaptically or by current injection. These effects were reversibly blocked by APV, and resolved after washing with Ringer.

This work was supported in part by the Department of Veterans Affairs (VTC) and by a grant from the New York State for Lyme Disease research (JMR).

**490.4**


During the course of an unrelated study we made the unexpected observation that the injection of small amounts of kainic acid in the deeper strata of the anterior third of the superior colliculus induced an acute and transient phase of rhythmic whisker movements. Since the vibrissae are extensively represented in this part of the superior colliculus and its neurons control them, the unexpected findings demand further investigation and as ascending projections we believe that this observation may be of some interest.

Nico Lang-Evans were anesthetized with 350 mg/kg chloral hydrate. Two to four unilateral injections of 0.5 ml kainic acid (3.52 mM) were made at 2-5 min intervals into the intermediate and deep strata. Within 1-3 min of the last injection of a 2-5 Hz rhythmic movement was seen. Although we have found a tendency for the vibrissae to be confined to the ipsilateral site, some animals injected the contralateral nostril. No other movements could be observed during the following 1-2 min. Neither the EEG nor bipolar recordings from the hippocampus indicated typical seizure activity nor could we detect any hippocampal neuronal damage in rats sacrificed within one hour of the excitotoxic injections.

At this time we cannot entirely rule out the possibility of an abortive limbic seizure due to the susceptibility of the hippocampus to kainic acid action “at a distance.” On the other hand, the possibility should not be completely discounted. Thus, we have confirmed that induced rhythmic reflects the transient activation of glutamatergic kainate receptors of tectal neurons. (Supported by NS20626)
ROLE OF EXCITATORY AMINO ACIDS IN THE NEURAL CIRCUIT 
MEDIATING THE ACUSTIC STARTLE REFLEX. M.J.D. Misranderino, N.M. Bach, R.F. Spiegel, and M. Davis, Dept. of Psychiatry, Yale Univ. Sch. of Med., 54 Park St., New Haven, CT. 06508

Acoustic startle is a short latency reflex elicited by a brief auditory stimulus. The neural circuit mediating the acoustic startle reflex is thought to consist of: the ventral cochlear nucleus (VCN); the paralemniscal area, an area just medially and ventrally to VCN; the nucleus reticularis pontis caudalis (RPC); and motor neurons of the lumbar spinal cord. Infusion of selective competitive excitatory amino acid antagonists into these sites causes significant and consistent increases in the magnitude of the startle response.

Specifically, local infusion into the VCN showed that while the non-NMDA antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) attenuated the startle reflex, the NMDA-specific antagonists AP5 (D-2-amino-5-phosphonovaleric acid) and AP7 (5-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid) were without effect. In the VLL of RPC, NMDA and non-NMDA antagonists were equipotent in blocking startle; however, RPC showed a much greater sensitivity to these drugs than other startle circuit sites. Intracerebroventricular infusion of AP5 and CNQX into the lumbar cord region again showed both these compounds to be about equipotent in attenuating whole-body startle. A finer analysis of acoustically elicited startle latency electromyographically from the quadriceps femoris complex in the hindlimbs showed that intracerebral CNQX (preferentially blocked an early, short latency (<50 ms) EMG component of startle, while AP5 preferentially blocked a late (>150 ms) component.

These findings indicate that excitatory amino acids may mediate or modulate the acoustic startle reflex at each nucleus within its neural pathway, and suggest that the temporal and relative contribution of NMDA and non-NMDA receptors, and their sensitivity, may vary as a function of site.

DESCENDING AND SEGMENTAL GLUTAMATIC INPUTS MEDIATE TWO DISTINCT EPSPs IN RAT SYMPATHETIC PREGANGLIONIC NEURONS. E. Shen, N. Ho and H. O. Dom. Dept. of Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153

Intracellular recordings were made from antidromically identified sympathetic preganglionic neurons (SPGns) in thin (500 μm) transverse neocortical (12-22 mm) and mesencephalic (4-5 mm) spinal cord slices. Electrical stimulation of dorsal roots elicited in SPGns a long latency (1-5 ms), relatively slow rising and decaying EPSP, referred to as DR-EPSP. Hyperpolarization and depolarization from the resting potential of about -60 mV reduced and increased the DR-EPSP. The response was potentiated in Mg-free solution; often giving rise to a burst of spike discharges. DR-EPSPs in the presence or absence of Mg ions were suppressed by the NMDA receptor antagonist AP5 (1-10 μM) but not by APV and ketamine (D-20 μM) but resistant to the KA/OA receptor antagonists DMOX and CNQX (1-10 μM). Stimulation of lateral funiculus evoked a short latency (<1 ms), relatively fast rising and falling EPSP, the LF-EPSP. The responses were increased by hyperpolarization and reduced by depolarization. Mg-free solution had no appreciable effects on LF-EPSPs. Contrary to the DR-EPSP, the LF-EPSP was antagonized by SNX0 and CNQX but not by APV and ketamine. The results indicate that SPGns receive two glutamatergic inputs: a primary synaptic connection from dorsal roots and a monosynaptic input from supraspinal neurons via lateral funiculus. Activation of these two inputs elicits two distinct EPSPs mediated by NMDA and non-NMDA receptors, respectively. (Supported by NS83170.)

HOW NMDA AND NON-NMDA RECEPTORS CONTRIBUTE TO RESPONSES IN VISUAL CORTEX. N. W. Daw and K. Fox, Dept. Cell Biol., Washington Univ. Med. Sch., St. Louis, MO 63110

We have observed the effect of glutamate agonists and antagonists on contrast sensitivity in cats and primates. In both species, NMDA increases the slope of the curve without changing the levels of contrast that give threshold and saturation, while APV reduces the slope and quinpirole shifts the curve upwards.

We have developed a model that accounts for these results using parameters for the cell biology of glutamate receptors from the hippocampus. The NMDA channel conductance has a Boltzmann voltage dependency with exponent -0.07V, while glutamate binds at non-NMDA and NMDA receptors with Hill coefficients of 1 and 2 respectively. Both the release of glutamate from terminals in the cortex is assumed to be related to contrast by a hyperbolic tangent formula, C/Cc + K, where C is contrast and K is a constant.

Several conclusions can be drawn. NMDA receptors contribute to the visual response multiplicatively, while non-NMDA receptors contribute additively. The NMDA receptor is active at low light as well as high contrasts, accounting for a constant percentage of the response at all contrasts. It does not act as a switch that turns on calcium inflow at some particular level of input. Consequently NMDA receptors contribute to the visual response in both adult and developing visual cortex in a graded fashion.

ELECTROPHYSIOLOGICAL EVIDENCE THAT A TAURINE-LIKE AMINO ACID IS THE NEUROTRANSMITTER AT A FAST EXCITATORY SYNAPSE. Peter A. Anderson and H.G. Tripathi-Rosenthal. Whitney Laboratory and Depts of Physiology and Neuroscience, Univ. of Florida, St. Augustine, FL 32084

Neurons in the motor nerve net of the jellyfish Cerata capillata are connected by fast relay synapses. The average delay between the peak of the presynaptic action potential and the peak of the EPSP is 1 ms, suggesting that the neurotransmitter at these synapses cannot be peptidergic, as appears to be the case in many cnidarian synapses. Previous work involving applications of 25 putative transmitter to exposed synapses failed to evoke any activity consistent with the normal EPSPs at these synapses. However, applications of taurine and β-alanine, but not alanine, and some, but not all analogs, evoked reproducible depolarizations of the cells. These were associated with a conductance increase. The reversal potential of the response averaged +6.8 mV, close to the average reversal potential of EPSPs at these synapses (+4 mV). This physiological evidence for a role of a taurine-like transmitter is supported by finding that neurons from this animal contain large amounts of taurine, β-alanine, alanine and GABA. If release studies, currently underway, confirm the role of a taurine-like neurotransmitter at these synapses, this will constitute the first convincing demonstration of their role as neurotransmitters.

Supported by NSF grant BNS-8805885.

ELECTRICAL ACTIVITY IN THE SUPRACHIASMATIC NUCLEUS, EVOKED BY OPTIC TRACT STIMULATION, CAN BE RECORDED USING VOLTAGE-SENSITIVE DYES. H. Komura*, A.I. Obaid, and B.M. Salberg. Dept. of Physiology, Univ. of Pennsylvania School of Medicine, Phila., PA 19104-4055

In an effort to understand the functional organization of the suprachiasmatic nucleus (SCN), we have used a system for Multiple Site Optical Recording of Transmembrane Voltage (MSPOT) and the potentiometric grays-emosual dye RH 155 to monitor electrical events that follow stimulation of the retinohypothalamic pathway. Coronal slices, 300 μm thick, were cut at the level of the optic chiasma of adult mice, pinned for 30 minutes in a 0.2 mg/ml solution of dye, and imaged onto a 124 element photodiode array using a 10X, 0.4 nA, objective. Brief stimuli (100-200 ms) were delivered to the optic tract by means of a bipolar electrode. Each photosensitive spot monitored current-voltage relationships that intensity which were proportional to the potential changes of the plasma membranes in its 100 μm square receptive field. These extrinsic optical signals derived mainly from retinal ganglion cell axons and neurons and exhibited waveforms having at least two components whose relative sizes depended upon location within the slice as well as the origin of the slice on the rostral-caudal axis. The first component was very fast, TTX-sensitive, and appeared to be dominated by the axonal spike. The slower portion of the optical signal had a wider distribution in more rostral slices. It was sensitive to high (11 mM) MgCl2, and to blockers of glutamnergic transmission such as kynurenic acid (1 mM), and a spatial organization of these effects could be discerned. 6-Cyano-7-nitroquinoxaline-2,3-dion (CNQX) eliminated the slow component at a concentration of 20 μM. These findings suggest that the second component of the absorption change reflects postsynaptic voltage changes in SCN neurons.

Supported by NS 16824 and a grant from the Government of Japan to HK.

CHARACTERIZATION OF GLUTAMATE RECEPTORS AND THEIR MODULATION BY VASOPRESSIN IN RAT HYPOTHALAMIC MAGNOCELLULAR NEUROSECRETORY CELLS (MNCs). B. Nis and C.W. Bourguet Centre for Research in Neuroscience, MGH, McGill University, Montreal, Quebec, Canada H3G 1A4 Glutamate receptor antagonists have been shown to block evoked excitatory synaptic transmission in the supraoptic nucleus (Gribkoff et al., J. Neurophysiol., 1990). Experiments on ventral septal neurons have previously shown that glutamate evoked firing can be modulated by vasopressin (VP) (Disturnal et al., CHP, 1997). Since 50% of suprachiasmatic MNCs release VP, we have characterized the glutamate receptor subtypes on suprachiasmatic neurons and examined the effects of VP on their activation by selective agonists. Forty-eight suprachiasmatic MNCs were recorded using a single electrode voltage clamp techniques. Bath applications of DNQX, quiniquale, kainate or glutamate consistently induced a dose-dependent, TTX-sensitive inward current or membrane depolarization (r=1 at rest). The relationship of the MNC-induced current revealed a Mg-dependent, negative resistance between -100 to -5.9mV (n=9), in contrast, currents evoked by quiniquale and kainate displayed linear relationships. MNCs induced responses were reversibly antagonized by APV (10 to 40 μM; n=5), while quiniquale responses were selectively blocked by CNQX (10 to 20 μM; n=4), suggesting that both NMDA and non-NMDA receptors are present on MNCs. Bath application of vasopressin (10 to 100 μM) reversibly attenuated MNC induced responses (50 to 100%; n=3) in a concentration-dependent manner. In contrast, quiniquale induced responses in the same cells were unaffected or enhanced by VP (n=5). The modulatory effects of VP may be relevant to the physiological control of suprachiasmatic neurons as well as their peculiar resistance to glutamate toxicity. Supported by MRC, FCAR and FRSQ.

Nordenergic locus coeruleus (LC) neurons receive prominent excitatory amino acid (EAA) and GABA inputs from their two regions that project to the rostral and caudal medulla (Enn Fail, et al., Neurosci., 6: 344-6, 1980), and the characteristic activation of these cells by sciatic nerve stimulation is mediated by GABA inputs in LC. Using this system, we examined possible GABAergic modulation of synaptic EAA-mediated transmission in vivo. Extracellular recording was combined with local microinjection of drugs into LC. Direct application of GABA antagonists (bicuculline methiodide [BIC] or 50 or 500 μM) onto LC neurons enhanced their sensory responsiveness by 233% (p<0.04, n=7). With the increased responsiveness was due to the long-lasting expression of a new, N-methyl-D-aspartate (NMDA) receptor-mediated component of the sensory response, as it was completely blocked by infusions of the NMDA antagonist, 2-amino-5-phosphonopentanoate (AP5). This action of BIC was neither mimicked by other GABA-A (picrotoxin or penicillin) or GABA-B (3-hydroxyaspartate) antagonists, nor by agents that directly depolarize LC neurons (vasoactive intestinal polypeptide or carbachol). This BIC-potentiated response component was eliminated by direct application of the neurotransmitter GABA.

These results indicate that BIC, acting at a novel site, unmasks NMDA receptors which can be activated by sensory stimuli. This new site may be a point of convergence where interactions between two major neurotransmitter systems potently modulate signal transmission in the brain.

Supported by HHRI grants NS24699 & DA026214.

ISOFLURANE DEPRESSION OF CA2+ COMPONENT INVOLVED IN NKDA AND GLUTAMATE ACTIONS ON CORTICAL NEURONS. H. El-Beheiry, E. Pull and K. G. Bainbridge, Dept. of Anaesthesia, Pharmacology & Therapeutics, and Physiology, Univ. of British Columbia, Vancouver, B.C., V6T 1W3, Canada.

A decrease in synaptic excitation has been suggested as a mechanism of action of general anaesthetics. The effects of isoflurane were studied using intracellular recording techniques and microspectrophotometric measurements of intracellular Ca2+ concentration (Ca2+2) with a Fura-2 probe in neocortical and hippocampal neurons. In the presence of external Mg2+ (2 mM), isoflurane depressed, in a dose-dependent manner, the depolarizations and associated conductance changes evoked by ionophoretic application of NKDA, and glutamate (Glut). Anaesthetic administrations in the lower dose range (IL5-1,5MAC) had no effects on the passive membrane properties of the neocortical neurons. In higher doses, isoflurane induced slow hyperpolarization (3-5 mV) as well as increased input conductance (40%). In hippocampal neurons, isoflurane attenuated, in a dose-dependent manner, the increases in [Ca2+]2 evoked by bauxis injection of Glut or NKDA under conditions that favoured activation of the NKDA and quisqualate-receptor subtypes. Verapamil perfusion reduced the augmented voltage-dependence increases of [Ca2+]2 induced by Glut application. The effect of isoflurane were additive with those of verapamil. It is suggested that isoflurane suppresses the depolarizations induced by NKDA and Glut by actions on receptor- and voltage-dependent Ca2+ influx or on processes for intracellular Ca2+ mobilization activated by Glu-receptor interactions.

GLYCINE MODULATES EXCITATORY AMINO-ACID-INDUCED EXCITATION OF RAT CEREBELLAR PURKINJE CELLS IN VIVO. J.G. Netzband, J.C. Strahle and H.K. Strahle, Departments of Physiology and Neurology, Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX 79430.

The action of glycine on excitatory amino acid-induced excitation of cerebellar Purkinje cells was investigated in urethane-anesthetized, adult, male cerebellar granule neurons in vivo. The effects of glycine on action of glycine on a low dose of glycine (5 nM) in these cells, showed no augmentation of excitation. In higher concentrations of glycine, the effects of glycine were less effective in potentiating NKDA, and in several cells, the effect was reversed to one of attenuation. These results suggest that glycine elicits a non-selective inhibition of excitation onto Purkinje cells, and that it also selectively potentiates responses to NKDA. This selective action on NKDA is in agreement with data from other areas of the nervous system. The results show that the NKDA potentiates an allosteric glycine binding site and lends further support to the hypothesis that functional NMDA receptors exist on adult cerebellar Purkinje cells supported by NS19296 and the Tr. Adv. Res. Prog., Grant 01067-025.

THE BLOCKADE OF POTASSIUM CONDUCTANCES BY EXCITATORY AMINO ACIDS IS MEDIATED BY A SUBTYPE OF QUİSQUATE RECEPTOR. S. Charpak, T. Krögel, S. Thompson, R.H. Gähwiler, Brain Research Institute, Univer. of Zürich, CH-8029 Zürich (Switzerland).

Recently we reported that cysteine could block the slow spike afterhyperpolarization (AHP) of hippocampal CA3 pyramidal neurons (Charpak et al. Soc. Neurosci. abstr. 374.13, 1989). In the present work, we characterized the endogenous agonists, receptor distributions, and the effects of various quisqualate receptor antagonists on this response.

Organotypic cultures of rat hippocampus were prepared as described previously. After 2-5 weeks in vitro, the cultures were superfused with a balanced salt solution containing 1 mM TTX and single-electrode voltage-clamp techniques were used to record currents from 100-500 μM of quisqualate and by agonists of the quisqualate metabotropic receptor such that Tris-1-amino-cyclooctyl-1,3-dicarboxylic Acid. Combined intracellular and eura-2 recording in this slice preparation was used to measure the whole-cell tight seal technique. We observed miniature spontaneous excitatory synaptic currents (meps) in most neurons investigated. Low-frequency stimulation of the afferent input to these areas produced evoked excitatory synaptic currents (epscs). The synaptic currents of both meps and epscs present in addition to a main fast decay a slower component (20-300 msec duration) relevant at holding potentials more depolarized than -55 mV, due to the relief of the Mg2+ blockade of NMDA-activated receptor-channels. Negative allstERIC modulators for the glycine site located on the NMDA receptor domains such as 7-Cl-kynurenic acid abolished the slow component of the epsc. Toneation of the excitatory input to dentate gyrus granular cells and CA1 pyramidal neurons produced in most cases long-lasting facilitation of the epscs and in some cases also of the meps if combined with depolarization of the postsynaptic neurons in the presence of picrotixin. 7-Cl-kynurenic acid prevented the induction of this facilitation. These results demonstrate a role for the glycine regulatory site in the excitatory synaptic transmission in the hippocampus.

This work is supported by an NHI grant # PO1 NS-78130.


Excitatory synaptic transmission in the dentate gyrus granular neurons and CA1 and CA3 pyramidal neurons of the rat hippocampus in this slice preparation (Edwards et al. Pfluger Arch. 414: 600, 1989) was studied by mean of the whole-cell tight seal technique. We observed miniature spontaneous excitatory synaptic currents (meps) in most neurons investigated. Low-frequency stimulation of the afferent input to these areas produced evoked excitatory synaptic currents (epscs). The synaptic currents of both meps and epscs present in addition to a main fast decay a slower component (20-300 msec duration) relevant at holding potentials more depolarized than -55 mV, due to the relief of the Mg2+ blockade of NMDA-activated receptor-channels. Negative allstERIC modulators for the glycine site located on the NMDA receptor domains such as 7-Cl-kynurenic acid abolished the slow component of the epsc. Toneation of the excitatory input to dentate gyrus granular cells and CA1 pyramidal neurons produced in most cases long-lasting facilitation of the epscs and in some cases also of the meps if combined with depolarization of the postsynaptic neurons in the presence of picrotixin. 7-Cl-kynurenic acid prevented the induction of this facilitation. These results demonstrate a role for the glycine regulatory site in the excitatory synaptic transmission in the hippocampus.

This work is supported by an NHI grant # PO1 NS-78130.
SYNAPTGENESIS AND ENHANCED SENSITIVITY TO NMDA FOLLOWING CHRONIC ADMINISTRATION OF PCP.

W.L. Brooks, T.G. Petit and J.C. LeBoutillier* Depts. of Pharmacology and Physiology, Univ. of Western Ontario, Scarboro, Ont., Canada, Mic 1L4.

Previous research in our laboratory has found that chronic administration of the non-NMDA antagonist phencyclidine (PCP) inhibits developmental synaptogenesis. The current research attempted to determine the effects of terminating a 14-day subcucuianous injections of 10 mg/kg PCP for two weeks (the period of maximal neocortical synaptogenesis). The rats were sacrificed 1, 2, 3, 15 and 25 days after the last injection of PCP and cortical sections were processed for electron microscopy. Analysis of the molecular layer of occipital cortex revealed an initial (21) drop in total number of synapses followed by a period in which total synapses exceeded control values (P<.05). This period of synaptogenesis coincided with an enhanced sensitivity to NMDA induced seizure activity. These results provide further evidence of a relationship between NMDA sensitivity and the ongoing rate of synaptogenesis.

PRESYNAPTIC GLUTAMATE RECEPTORS MODULATE NEUROTNSER RELEASE FROM SYNAPTOSOMES.

James K.T. Wang and Vijay Thakur* Tufts University School of Medicine, Boston, MA 02111.

The existence of presynaptic glutamate receptors and the question of which of the glutamate receptor subtypes regulates transmitter release, was addressed in this study. We labeled synaptosomes from adult rat olfactory bulbs with [3H]diperoxiphine, [3H]dipone, or [3H]CGBABA, and assayed transmitter release in a superfusion apparatus. Glutamate (10 mM) increased the basal release of the two catecholamines by 50% within 1-2 min, and did not have any significant effect on the K+ depolarization evoked release. The enhanced basal release was dependent on extracellular Ca++, and was blocked by the non-NMDA subtype selective antagonist CNQX (10 mM) but not by the NMDA subtype selective antagonist APV (10 mM). Quisqualate (100 mM) had similar effects as glutamate, but kainate and NMDA did not. Glutamate also increased both the basal and the K+ depolarization-evoked efflux of [3H]CGBABA. However, these effects were not blocked by either CNQX or APV, were not mimicked by AMPA, kainate, or NMDA, and were not dependent on extracellular Ca++. These data suggest that in olfactory bulb synaptosomes there are presynaptic glutamate receptors, possibly of the non-NMDA quisqualel/AMPA subtype, that modulate catecholamine release. In contrast, the effects of glutamate on GABA efflux may not involve the Ca++-dependent, releasable pool of the transmitter. (Supported by the Pew Charitable Trust)

CEREBRAL SYNTHESIS AND RELEASE OF KYNURENIC ACID.

K.A. Swarta, M.C. Hardon, A. Freese and M.D. Berlin Program in Neuroscience, Harvard Medical School, and Mass General Hospital, Boston MA 02115.

Kyurenine is an endogenous antagonist of excitatory amino acid (glu or Asp) receptors and may therefore influence important physiologic and pathologic processes. The release of intracranially synthesized kyurenine acid into the extracellular fluid (ECF), where it may act as an EA receptor agonist, has not been established in vivo. Furthermore, kyurenine acid synthesis from physiologically supplied precursors has not been demonstrated in vivo or in vitro. We studied the synthesis and release of kyurenine acid in rat striatal microdialysis in vivo using intracranial microdialysis and HPLC fluorescence detection. The basal ECF concentration of kyurenine acid in the rat striatum was 17.1 ± 1.6 nM. Peripherally administered labeled precursor of kyurenine acid, L-kyurenine, resulted in marked dose dependent increases in striatal ECF concentrations of kyurenine acid, peaking at 2.5 hrs. The highest dose of L-kyurenine (1000 mg/kg) administered peripherally, resulted in a 108 fold increase in plasma kyurenine acid levels and a 37 fold increase in cerebrospinal fluid levels. Peripheral administration of kyurenine acid, at a dose that caused plasma levels to increase 430 fold, resulted in only 4 fold increases in striatal ECF concentrations. The precursor responsiveness of striatal ECF kyurenine acid to peripherally infused L-kyurenine was blocked by the central application (via the dialysis probe) of iNAM amanitoxin acid, an inhibitor of the immediate synthesis enzyme for kyurenine acid, kynurenine aminotransferase. Administration of L-tryptophan, was less effective than L-kyurenine in increasing ECF kyurenine acid concentrations, and did so at a considerably later time interval (6 hrs.). Infusion of L-kyurenine (100 mM), but not L-tryptophan (100 mM), through the dialysis probe, dexamethasone increased striatal ECF concentrations of kyurenine acid. The conclusions drawn from the present study are that: i) kyurenine acid is present in ECF within the central nervous system (CNS), ii) the CNS can release kyurenine acid from, iii) the majority of CNS kyurenine acid synthesis results from the transport of L-kyurenine across the blood brain barrier and iv) ECF concentrations of kyurenine acid can be drastically increased by pharmacological manipulation of precursor levels.

APNEA PRODUCED BY MICROINJECTION OF KYNURENIC ACID INTO THE CAUDAL SUBRETROFACIAL AREA OF THE VENTROLATERAL MEDULLA IN THE CAT IS DUE TO BLOCKADE OF BOTH NMDA AND NON-NMDA EXCITATORY AMINO ACID RECEPTORS.


We recently reported that bilateral microinjection of kyurenine acid (KYN; 12.5nmol in 50nl) into the caudal-subretrofacial area produces apnea in chloralose anesthetized cats (Neurosci. Abstr. 15:100, 1989). The purpose of the present study was to determine which subtype(s) of excitatory amino acid (EA) receptor is(are) involved in maintaining the normal breathing pattern at this site. For this purpose, antagonists of NMDA, L-glutamate, and AMPA were microinjected into the caudal-subretrofacial area of the medulla of 6 cats. The apneic response to microinjection of KYN (50nl) into this area was reduced (approx. 3 mm ratio to baseline, 4 mm lateral to midline and 1.5 mm below the ventral surface) when monitoring arterial pressure (BP), heart rate (HR), tidal volume (TV) and respiratory rate (f) in chloralose anesthetized cats. In the case of KYN, these doses were studied (0.25nmol, n=3; 0.75nmol, n=3; 2.25nmol, n=2). All three doses produced similar decreases in Vt (-12±3, p<0.05, -10±3, p<0.05), and -16±5nl/min respectively, and increases in (f+14±5, p<0.05, -10±3, p=0.05, and -12±5nl/min respectively). Non-NMDA antagonists exhibited an in contrast, microinjection of CNQX (0.05nmol, N=3) resulted in only a small increase in f (+17±6, p<0.05) and -16±5nl/min with no significant change in Vt. A combination of CNQX and L-glutamate (L-Glu; 2.25nl/min) increased f (+24±7nl/min, p<0.05) and an increase in f (-11±4nl/min, p<0.05). Three of the five animals tested exhibited apnea. None of these drugs produced significant effects on BP or HR. These results indicate that the major EA drive to respiratory neurons in the caudal-subretrofacial area involves activation of NMDA receptors but excitation of non-NMDA receptors may also play a crucial role in maintaining respiration. Supported by USPHS grant 1P01 NS28130.
PHOSPHATE-ACTIVATED GLUTAMINASE (PAG) INHIBITORS ABOLISH GLUTAMATE-IMMUNOREACTIVITY IN THE RAT CEREBRAL CORTEX. F. Conti, M. Fabrizi, A. Miconi* and C. Sagliotti*. Institute of Human Physiology, University of Ancona School of Medicine, 1-6031 Ancona (Italy).

Conversion of glutamine (Gln) to glutamate (Glu) can be catalysed by phosphate-activated glutaminase (PAG) plays an important role in the formation of transmitter Glu. In the experiments reported here we have studied the effects of two PAG inhibitors, NS27751 (MA) and N-enamylasamidae (NEM) on the pattern of glutamate-immunoreactivity (IR) in the rat cerebral cortex.

1) MA (1mM) and NEM (1mM) were applied either intraperirenalaneously (0.2 µl) or topically on the cerebral cortex. After 30 min incubation in 4% paraformaldehyde, sections were then processed for immunohistochemistry (Conti et al. J. Neurosci. 1987-1988). Controls included: sham injections, absorption of physiological saline, and NEM staining of adjacent sections to rule out aspecific effects, and MA and NEM solutions in physiological saline did not change the pattern of Glu-IR.

2) Since PAG activity is involved in the formation of transmitter Glu, these results provide further evidence that Glu-IR observed in the cerebral cortex is related primarily to the transmitter pool of Glu.

ELECTRICAL-STIMULATION INDUCED RELEASE OF EXCITATORY AMINO ACIDS, INCLUDING SULPHUR-CONTAINING COMPOUNDS, FROM ACUTE HIPPOCAMPAL SLICES. J.M. Kimelberg, M.C. Fabritiis and K.O. Do Brain Research Inst., Univ. of Zurich, Switzerland.

Excitatory amino acids (EAAs) have been suggested as possible neurotransmitters in the hippocampus, and have been shown to be released by high-K⁺ stimulation in a Ca²⁺-dependent manner. To identify putative neurotransmitters released from specific hippocampal areas, however, it is necessary to stimulate more selectively. The present study examined the release of amino acids from acute 480mM hippocampal slices prepared from adult SWV-50 rats in the presence of glutamate receptors. In a subculture type recording chamber, a 30mm diameter cannula was placed 50-60mm from the CA1 stratum radiatum, to collect 12 consecutive 1-min fractions of superfushate at a rate of 200µl/min and was placed microelectrode (4.5 mV, at 50Hz, 100usec pulse duration and an intensity which induced a population spike of maximal amplitude recorded in CA1 stratum pyramidale. Analysis was performed on the EPLC using derivatization with o-phthalaldehyde, using a linear gradient of 0.03M acetic buffer (pH 7.0) and acetone. Consistently, aspartate (Asp) levels were increased (from 0.05±0.02 to 1.55±0.71 pmol/10³ slices) upon stimulation, while extracellular, valine or phenylalanine showed no changes. An increase in glutamate levels was observed occasionally. In some experiments, low levels (approx. 0.2 pmol/pulse) of homocysteic acid (HCA) or cysteine sulfinic acid (CSA) were detected only during or following stimulation. Although high K⁺-induced release of HCA and CSA from hippocampal tissue has been shown previously, these results are the first to indicate a release of these sulphur-containing EAAs upon electrical stimulation. The present results suggest that Asp, HCA and CSA play a role in synaptic transmission in the Schaffer collaterals.


We have used in situ hybridization (ISH) to map the distribution of mRNAs coding for the glutamate-metabolizing enzymes, glutamine synthetase (GS), glutaminase (GA) and glutamate dehydrogenase (GHD) in the adult rat brain. ISH was carried out on frozen brain sections using synthetic 48-mer oligonucleotide probes directed against regions of the coding sequence for GS, GA and GA.

GS mRNA was located to glial cells in both white and grey matter. High density of labelling was associated with the Virchow-Robin space. A larger cell layer, in particular the entorhinal cortex, pontine nuclei, olfactory bulb and GDN mRNA was found in both glial cells and neurons. The localization of the mRNAs was very similar to that of GA, while the glial distribution was similar to that of GS. The localization of the mRNAs was well the immunochemical distribution of cells labeled with antibodies against GS, GDN and GA.


Multiple lines of evidence implicate the excitatory amino acids, L-aspartate (L-asp) and L-glutamate (L-Glu) as chemosynthesizing afferent fibers. The objective of this study was to develop an experimental model to determine whether the EAA are released from primary afferents. Dorsal root ganglia (DRG) from 1 to 8 day-old rats were dissected on chopper and placed in Petri dishes for 7 to 10 days. After a 80 min equilibration period in Ringer's electrophysiological recording solution, the mean concentrations of EAA recovered during a 5 min interval were 1.2µM for L-Glu and 0.127µM for L-asp. Suppression of DRG ganglios of cultures with either 25 or 50 mM potassium resulted in a concentration-dependent release of both L-asp and L-Glu. The primary electrolyte of K⁺ however, failed to increase the release of EAA from cultures where DRG cell bodies were removed 72 hours prior to these experiments. These results demonstrate that EAA are released from mammalian primary afferent fibers, and in particular, released from small diameter primary afferents. Work was supported by NIH grant NS27775, USDA grant PL5-113, NIA grants DA 04190, DA 04090, DA 00124.


It has been reported that metabolites of arachidonic acid are essential for catecholaminergic release from the adrenal medulla and stimulate glutamate release from synaptosomes. This study examined the effects of nordihydroguaiaretic acid (NDGA), an inhibitor of the lipoygenase pathway, on the release of glutamate and aspartate from the Schaffer collateral-commissural-ipsilateral associative (SCIA) pathway in area CAI of the rat hippocampus. The release of glutamate and aspartate from superfused slices of the CA1 area (excluding stratum lacunosum-moleculare) by exposing the slices to 1 min pulses of 50 mM K⁺. Under our conditions nearly all the release of glutamate and aspartate is Ca²⁺-dependent and it originates predominantly from the SCIA pathway. The release of 100 µM NDGA to the superfusion medium released endogenous glutamate and taurine from the slices. No aspartate release was detected. NDGA-evoked amino acid efflux was not Ca²⁺-dependent. When the slices were depolarized with elevated K⁺, NDGA depressed the evoked release of both glutamate and aspartate by about 80%. Indomethacin, an inhibitor of the cyclooxygenase-pathway, did not affect glutamate or aspartate release. These findings support a crucial role for lipoygenase products of arachidonic acid metabolism in the synaptic release of excitatory amino acids. (Supported by NIH grant NS 16084.)

ALTERATIONS IN REGIONAL KINETICS OF PHOSPHATE-ACTIVATED GLUTAMINASE WITH REGARDS TO AGE. D.R. Wallace and R. Davson, University of Florida, Dept. Pharmacodynamics, Gainesville, FL 32610.

Phosphate-activated glutaminase (PAG-L-glutamine amidohydrolase EC 3.5.1.20) hydrates glutamate and thus forms both glutamate and taurine. We have previously shown that a difference in regional ammonia inhibition exists by 8 month and 30 month old male Fischer-344 rats. The present study addresses the kinetics of PAG in the temporal cortex (TCo), striatum (STR), and hippocampus (HIPP) from 8 month and 30 month old F-344 rats. The basal activity (92 fraction in the presence of 0.5M glutamine and 10mM phosphate) was the highest in the TCo followed by the STR, and then the HIPP which had the lowest basal activity. Michaelis-Menten kinetics were done using substrate concentrations of 0.1-5.0mM glutamine in the presence of 10mM phosphate. The Km for PAG was unchanged in any of the regions from 8 month old rats. The values for Vmax displayed a similar trend as basal activity (TCo>HIPP>STR). Vmax was significantly reduced approximately 15% in the STR in comparison to the TCo. In the HIPP, both KmA and Vmax were significantly increased (approximately 30% and 25%) respectively with regards to age. There was no change in the HMPC level in the changes seen in the present study suggest that PAG is selectively altered in the aged brain, and that regional differences exist suggesting the existence of regional PAG substrates. (Work supported by a predoctoral fellowship [15(AG-3-2104) and a predoctoral fellowship [AG-00190-01] from the Center for the Neurobiology of Aging, Univ. of Florida.)
**401.9**

**GLUTAMATE IMMUNOREACTIVITY IN TERMINALS OF CORTICAL EFFERENTS AND DORSAL ROOT AFFERENTS.** R.J. Welberg and D.A. Bastiani. Dept. of Cell Biology & Anatomy, University of North Carolina, Chapel Hill NC 27599.

Results obtained with techniques combining anterograde tracers with post-embedding electron microscopic immunocytochemistry will be illustrated using cortical efferents and primary afferents as models. Two days after injections of lectin-conjugated HRP in cortex, peripheral nerves, or dorsal root ganglia, rats were sacrificed by intraaortic perfusion with 2.5% glutaraldehyde, 0.5% paraformaldehyde, and 0.2% picric acid in phosphate buffer (pH 7.2). Perpendicular sections were processed for post-embedding immunocytochemistry for glutamate and GABA using two different sizes of gold particles. Substantial numbers of glutamate- and GABA-labeled terminals were observed in both brain stem and spinal cord. Gold particles were concentrated over sympathetic vesicles and microchondria. Density of gold particles over anterogradely labeled terminals differed in terminals of different origins. The combination of these techniques with the retrograde tracer WGAapoHRP-Au, allows a fine-grain analysis of the chemical anatomy of neuronal microcircuitry.

**401.10**

**GLUTAMATE AND ACETYLCHOLINE ARE CO-LOCALIZED IN THE LATEROДORSAL TEGMENTAL AND PEDUNCULOPONTINE NUCLEI.** J.R. Clements and S.J. Grant, School of Life and Health Sciences, Dept. Psychology and Prog. in Neuroscience, Univ. Delaware, Newark, DE, 19716.

Studies of the pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei in the mesoponsopic tegmentum have emphasized the organization and projections of cholinergic neurons. Few studies have focused on the role of excitatory amino acids in these regions. Therefore, we decided to examine the distribution of glutamate-like immunoreactivity (GLI) in the rat mesoponsopic tegmentum.

GLI neurons were found extensively distributed throughout the mesoponsopic tegmentum. Some neurons were intercalated between cholinergic neurons in the LDT and LPT, as well as in adjacent non-cholinergic areas. GLI neurons in the LDT and LPT were similar in morphological type and size to cholinergic neurons in these nuclei. Double labeling studies revealed a subpopulation of neurons in both the LDT and LPT which exhibited co-localization of GLI and ChAT-immunoreactivity. This suggests that some LDT and LPT neurons may contain both classical transmitters as well as previously described peptide co-transmitters. These data raise the possibility that PPT and LDT target sites receive combined excitatory amino acid and cholinergic inputs and that excitatory amino acids contribute to the functions of the LDT and PPT, including behavioral state control, locomotion and activation of midbrain dopamine neurons.

Supported by NIH (R35, NIMH (SIG), the State of Delaware and ICI pharmaceuticals (SIG).

**401.11**

**INCREASED LEVELS OF GLUTAMATE IMMUNOREACTIVITY IN THE AUDITORY NERVE ENDINGS OF THE DORSAL COCCHEAR NUCLEUS.** J.M., Kuz, R.H. Helfert, R.J. Wenthold and R.A. Aitschler, Kresge Hearing Research Program, Ann Arbor, MI 48109-1106, NIDCD, NIH.

Increased levels of glutamate are likely to be associated with excitatory amino acid terminals, either as a transmitter or part of the transmitter pool. Quantitative evaluation of immunogold labeling provides a sensitive technique to determine glutamate levels in synaptic terminals. We have used this technique with glutamate antiserum in the cochlear nucleus, where auditory nerve terminals are believed to use an excitatory amino acid as their transmitter. The number of colocalated gold particles in auditory nerve terminals compared to other terminals and glial elements in the dorsal cochlear nucleus is seen below:

![Glutamate immunoreactivity](image)

The increased immunoreactive labeling for glutamate in auditory nerve terminals suggests that such labeling may serve as a useful marker for excitatory amino acid terminals.

(Supported by NIH grant 2-R01-DC00383-04)

**401.12**

**IMMUNOCYTOCHEMICAL LOCALIZATION OF N-ACETYLATED ALPHA-LINKED ACIDIC DIPETIDASE (NAALADase).** B. Stauch, Sluscher, G. Tsai, and J.T. Coyle, Departments of Pharmacology and Neuroscience, The Johns Hopkins School of Medicine, Baltimore, MD 21205.

N-Acetylated alpha-linked acidic dipetidase (NAALADase) is a membrane-bound metallopeptidase that cleaves glutamate from the endogenous neuropeptide N-acetyl-aspartyl-glutamate (NAAG). We have purified rat brain NAALADase activity to apparent homogeneity, and have raised specific anti-NAALADase antibodies in guinea pig (Stauch et al., submitted). Immunocytochemical studies show intense NAALADase-immunoreactivity (NAALADase-IR) in several structures previously shown to contain NAAG-immunoreactivity (NAAG-IR), including the globus pallidus, entopeduncular nucleus, substantia nigra, hippocampus, reticular thalamic nuclei, medial and lateral geniculate, lateral habenular nuclei, periaqueductal gray, molecular and granular cell layers of the cerebellum, spinal trigeminal nucleus, substantia gelatinosa, lateral cervical nuclei, and intermediolateral cell column. Within these structures, immunoreactivity was distributed throughout the neuropil; no cell bodies were stained, even after colchicine treatment. The only exception was a subpopulation of cerebellar granule cells whose cell soma stained intensely. In addition, NAALADase-IR was observed in several fiber tracts, including the stria terminals, fornix, solitary tract, and corpus callosum; these fiber tracts also contain NAAG-IR. The co-localization of NAADase-like and NAAG-IR within the same neural structures supports the hypothesis that NAALADase is responsible for the catabolism of NAAG in vivo.

**401.13**

**Monoclonal Antibodies to N-Acetyl-Aspartate.** M.L. Simmons, C. Frongillo*, and J.T. Coyle. Dept. of Neurosciences, The Johns Hopkins University School of Medicine, Balto., MD 21205.

N-acetyl-aspartate (NAA) is found in high concentrations in all areas of the brain, but is undetectable in non-nervous tissue [Tallan, et al., 1956]. NAA and glutamate are produced as a result of the enzymatic degradation of N-acetyl-aspartyl-glutamate (NAAG), a putative neurotransmitter/messenger [Robinson, et al., 1987]. However, current information suggests that NAA may have functions unrelated to NAAG. For instance, analysis by HPLC has shown that NAA is present at nearly 10 times the concentration of NAAG, and is more homogeneously distributed than NAAG [Koller, et al., 1984].

Monoclonal antibodies to NAA were produced to further study its role in the brain.

Spleen lymphocytes from a mouse immunized with NAA conjugated to thyroglobulin by carbodiimide were fused with P3X63-Ag8 mouse myeloma cells. The product was a monoclonal antibody IgG2a/K) antibodies specific for conjugated NAA. Seven percent cross-reactivity with conjugated NAAG was observed only at high antibody concentrations; no cross-reactivity was observed with conjugated N-acetyl-glutamate or aspartate. Precipitation of the antibody with 0.5 mg/ml conjugated NAA blocked immunoreactivity 97%, while precipitation with conjugated NAAG and free NAA had no effect. Preliminary immunocytochemistry has shown that NAA-immunoreactivity is localized in neurons and widely distributed throughout the brain. This monoclonal antibody exhibits a high degree of specificity for conjugated NAA and will be useful in elucidating the function of NAA in the brain.

**401.14**

**ABNORMAL EXCITATORY NEUROTRANSMITTERS IN HUMAN AND CANINE DEGENERATIVE MOTOR-NEURON DISEASES.** G. Tsai, J.C. Cork*, J.C. Hedreen, B. Stauch, Sluscher, L. Passani, J.D. Robinson, and J.T. Coyle. Dept. Neuroscience, Johns Hopkins Hospital and School of Medicine, Balto., MD, 21205.

There is increasing evidence that excitatory neurotransmitters may be involved in the pathogenesis of degenerative motor neuron disease (DMD). Accordingly, we have measured aspartate (Asp), glutamate (Glu), N-Acetyl-aspartate (NAA), and N-acetyl-aspartylglutamate (NAAG) in pathologically confirmed amyotrophic lateral sclerosis (ALS) and hereditary canine muscular atrophy (HCM). In punch samples of human ALS cord (C), and ALS gluta (G) were also decreased in the white matter. In the HCMD, Asp, and NAAG were reduced substantially in ventral horn, dorsal horn, and ventralis (40%, 44%, 50%, and 60%, -36% respectively). In motor cortex, Asp and NAAG decreased significantly in deep gray matter (-32% and -29% respectively). Asp (-40%) and Glu (-46%) were also decreased in the thamic cord all decreased significantly (-53%, -55%, -23%, and -24% respectively), whereas NAAG in the motor cortex. The motor cortex of the dogs was devoid of pathology which is limited to the lower motor neurons in the spinal cord. These findings support the excitotoxic hypothesis of DMD and are consistent with previous report of the elevated NAA and NAAG in human ALS CSF. Since NAA and NAAG are highly concentrated in motor neurons, they may play a role in the pathogenesis of DMD.

N-acetylaspartate (NAA) and N-acetylaspartylglutamate (NAAG), a putative neurotransmitter in neuronal tissue. NAA has been suggested as a precursor and/or metabolite of NAAG. The development of affinity-purified antisera against conjugated NAAG has permitted the investigation of its cellular localization in rodent nervous tissue. Now highly specific monoclonal antibodies against conjugated NAAG has been successfully produced (see abstract, M. Simons et al.). To further understand the immunoreactive relationship between NAA and NAAG in primates, we have exploited a double-staining immunocytochemistry technique for visualizing these two antigens in the same cell section of primate brain. Six Macaque monkeys were perfused with a combination of paraformaldehyde and carbodiimide. Immunocytochemical specificity of the antibodies was demonstrated by their differential blocking by conjugated NAAG and NAAG. Preliminary data indicate NAA and NAAG are co-localized in different structures of the monkey brain. Thus, neurons in motor and cingulate cortex, the nucleus of the diagonal band, septal nucleus, hippocampus, globus pallidus, substantia nigra, subthalamic nucleus, lateral geniculate, deep cerebellar nuclei, brain stem and spinal cord motor nuclei all contain NAAG and NAA immunoreactivity. However, NAAG immunoreactivity was more widely distributed than that of NAAG. These results are consistent with previous findings on regional concentrations of NAA and NAAG. Also, they support a functional relationship between NAA and NAAG.

491.17 PREFERENTIAL ASTROGLIAL LOCALIZATION OF KYNURETINE AMINO-TRANSFERASE IN THE RAT HIPPOCAMPUS. R. Schwartz, F. Du, V. Schmidt and E. Okuno, Maryland Psychiatric Research Center, Baltimore, MD 21228.

Kyuretine aminotransferase (KAT), the biosynthetic enzyme of kynurenic acid, was recently purified to homogeneity from rat brain, and its identity with brain KAT was established (Okuno et al., submitted). Using rabbit anti-rat KAT antibodies, we have now studied KAT immunohistochemistry in the normal rat hippocampus. Control experiments, including absorption of antibodies with pure enzyme, showed no immunoreactivity. KAT immunoreactivity was predominantly observed in glial cells. In general, these cells had 4-6 primary processes radiating from the cell bodies. They were present in all hippocampal subfields along the rostrocaudal axis. The hilus contained a higher density of KAT-immunoreactive glial cells than CA3 and CA1 regions, whereas the granule cell layer and the adjacent portion of the molecular layer of the dentate gyrus harbored only a few KAT-immunoreactive glial cells. As demonstrated by double-labeling with glial fibrillary acidic protein, the vast majority of KAT-immunoreactive cells appeared to be astrocytes. In addition, sporadic neurons containing KAT were also detected, mainly in strata oriens and pyramidale. These neurons probably belong to a subpopulation of interneurons. The organization of cellular elements containing KAT might be of relevance for the function of kynurenic acid in the hippocampus. (Supported by USPHS grant NS 16102).


The localization of kynurenic acid phospho-biosyntheseaserase (QPRT), the degradative enzyme of the endogenous excitotoxin kynurenic acid, was studied in the human neocortex by immunohistochemistry. In normal human brains, QPRT-immunoreactivity (QPRT-1) was detected in both glial cells and neurons. Glial cells containing QPRT-1 had different sizes and shapes, and were tentatively classified into three subpopulations. Most were medium-sized cells with oval or elongated perikarya. Small QPRT-1 neurons, often spheroid in shape, were particularly noted in a zone of the caudate nucleus adjacent to the lateral ventricle. A few large QPRT-1 positive neurons were also observed. The somatic and dendritic morphology of QPRT-1 neurons resembled that of aspyging neurons seen in Golgi preparations. The localization of QPRT in different populations of neocortical neurons suggests specific functional correlates, and may be relevant for the pathogenesis of basal ganglia disorders. (Supported by USPHS grant NS 28236).


1Faculty of Pharmacy, University of Toronto, Toronto, Ontario, M5S 2Z5; Laboratory of Neurosciences, National Institute on Aging, Bethesda, MD 20892; 2Dept. of Pharmacology & Toxicology, Queen's University, Kingston, Ontario, K7L 3N6.

Kainic acid receptors mediate fast excitatory synaptic transmission in the CNS. Although the distribution of kainic acid receptors has been studied in a number of species using receptor autoradiography, very little is known about their cellular and subcellular distribution. We have produced a monoclonal antibody against a kainate receptor purified from frog brain. This antibody recognizes a protein in rat and mouse that is brain-specific and is present at 90,000 dalton levels of mouse brain revealed that the Mr = 90,000 protein was not detectable in the hippocampus until postnatal day 7; in the cerebellum, immunoreactivity was not observed at postnatal day 7 but was very intense in adult tissue. The subcellular distribution of the immunoreactivity was determined by immunoblot analysis of purified subcellular fractions prepared from rat forebrain. The protein was not present in the soluble or myelin fraction but was present in mitochondrial and microsomal fractions and was highly enriched in glutaraldehyde-soluble membranes and synaptic junctional fractions. Lesion studies in which dexamethasone was microinjected into the amygdala resulted in a decrease in immunostaining in the hippocampus 30 days after injection. These results indicate that this monoclonal antibody recognizes a kainic acid receptor with a Mr ~ 90,000 and that this receptor is located postsynaptically on nerve cell membranes. Immunocytochemistry at the EM level confirmed these results. Supported by NSERC and MRC (Canada).

491.20 ONTOGONNY OF EXCITATORY AMINO ACID RECEPTORS IN RAT BRAIN. R.L. Makowiec, S.Y. Sakurai, J.B. Penney, and A.B. Young. Dept. of Neurology and Neuroscience Program, University of Michigan, Ann Arbor, MI 48109.

The ontrogenic profile and regional distribution of excitatory amino acid (EAA) receptors were examined using quantitative autoradiography. We measured binding in the outer cortex, striatum (GP), CA1, CA3, and subiculum of Imm. male Sprague-Dawley (G) and Imm. male Sprague-Dawley (DG) in 1-, 4-, 7-, 10-, and 14-day-old male rats. The NMDA receptor complex was labeled using [3H]MK-801, [3H]glycine and NMDA-sensitive [3H]glutamate binding. Ionotropic and metabotropic quisqualate receptors were labeled using [3H]AMPA and AMPA-insensitive, quisqualate-sensitive [3H]glutamate binding (AQG/MP), respectively. Postnatal day (PND) 1 to PND 14 [3H]MK-801 and [3H]glycine binding increased in CA1, outer cortex and striatum while in GP binding remained at low levels. NMDA-sensitive [3H]glutamate binding exhibited a different profile. In striatum, CA1 and CA3 binding peaked at PND 7 and remained constant to PND 14. Binding in GP decreased from PND 1 to PND 14. These data suggest that the various components of the NMDA receptor may be differentially expressed during development. [3H]AMPA binding increased from PND 1 to PND 14 in all regions examined except in CA3 where binding declined during development. Metabotropic binding (AQG/MP) peaked at PND 10 in striatum and outer cortex and then decreased at PND 14. In CA1 and DG, metabotropic binding increased during the first two weeks. In CA3, however, binding peaked at PND 1 and then decreased over PND 14. Interestingly, in GP the relative amount of metabotropic binding was high at PND 1 and remained at this level through PND 14. These results suggest EAA receptors have unique ontogenetic profiles and may be involved in a variety of developmental CNS processes. Supported by USPHS grant NS 16133.

The genetically dystonic rat (dt) exhibits a complex and progressive movement disorder initially detectable on post-natal days 9 to 10. No detectable morphologic alterations were noted by histological analysis of the brain. The sign of the drug response elicited by the quisqualate-sensitive glutamate binding were characteristic of the dystonic rat. Recent studies indicate the failure to elicit harmaline-tremor in the offspring of dystonic rats and the offspring of normal parents. The results suggest that the effect of the drug is not due to a failure to activate olivary neurons. The results suggest that the effect may be due to a defect in the olivary pathway, possibly due to a defect in the GABAergic system. The results also suggest that the effect may be due to a defect in the olivary pathway, possibly due to a defect in the GABAergic system. The results also suggest that the effect may be due to a defect in the olivary pathway, possibly due to a defect in the GABAergic system.
492.5

MU AND DELTA OPIoid ANGioSTIS QUIt ISOLATioN
INDUCED VOCALIZATIONS IN RAT PUPS. Susan E. Carden, Gordon A. Harr, Mycon A. Rofer. Columbus University, Physicians and Surgeons.

During the first three weeks of life, rat pups react to separation from the homecage, dam, and littermates by emitting ultrasonic vocalizations. In 10-day olds, these characteristic distress cries are diminished if either a social companion or morphine (0.125mg/kg) are provided. The opioid antagonist naltrexone reverses the quieting effect of either a social companion or diazepam as well as blocking morphine effects, suggesting a role for opioids in the modulation of early isolated distress.

Isolated 10-day old rat pups received intracranial injections of agonists specific to the mu, delta, or kappa opioid receptors, (DAGO (0.01ug - 0.0625ug), DPPE (0.3ug - 3.0ug) and U50-488 (1.0ug - 100ug). The rate of vocalization was monitored for 6 minutes. Pups were evaluated for sedation and each injection site was verified. Mu and delta agonists DAGO and B-CM decreased the number of distress cries in non-sedated animals, while the kappa agonist U50-488 left vocalization rates unchanged.

492.7

DIPRENORPHINE DRUG DISCRIMINATION: ASSESSMENT OF NALTREXONE AND ANALOGOUS SUBSTUTION. S. Smurthwaite and A.L. Riley. The American University, Washington, D.C.


Dorsett and Holtzman (J. Pharmacol. Exp. Ther., 237: 437-444, 1986) have recently reported that monkeys were able to discriminate between the opiate antagonist diprenorphine (HCl) and i vehicle. However, other opiate antagonists failed to substitute for the diprenorphine cue. Paradoxically, opiate agonists did substitute for diprenorphine. Because Geter and her colleagues (Neurosci. Abst., 15:248, 1989) have recently demonstrated generalization between diprenorphine and naloxone in naloxone-trained subjects within a taste aversion procedure, the present experiment reexamined drug discrimination learning with diprenorphine within this design. Specifically, every fourth day for 13 conditioning trials rats were administered diprenorphine (3.2 mg/kg) 15 min prior to saccharin ingestion. Following acquisition of the discrimination, both naltrexone (0.18 to 10 kg/mg) and naloxone (0.32 to 32 kg/mg) substituted for diprenorphine, while the opiate agonist morphine (3.2 to 10 mg/kg) did not. The present data are consistent with other work within the taste aversion design demonstrating generalization between the opiate antagonists.

492.8


Much less is known about sigma than phencyclidine (PCP) receptors because there are very few selective sigma ligands. The purpose of this study was to characterize the biological activity of a new class of sigma ligands: 9,10-ethanoanthracene-like compounds and related analogs, which interacted selectively with sigma receptors, but not PCP receptors. SC-48960 and SC-49574, two of the more potent sigma ligands in this series, had very weak affinity for alpha-, alpha-, beta-,adrenergic or dopamine receptors. SC-48960 had moderate affinity for muscarnic, serotonin, and dopamine, receptors, but SC-49574 had no affinity for any of these receptors. SC-48960 and SC-49574 did not produce any stereotyped behavior or ataxia in rats, but antagonized (+)-SKF-10,047-induced stereotyped behavior and ataxia. These results indicate that SC-48960 and SC-49574 are potent and selective sigma ligands capable of antagonizing some of the behavioral effects of sigma ligands.

492.9

CENTRAL ADMINISTRATION OF δ-CASOMORPHINS AFFECT ANTINOCICEPTIVE BEHAVIOR IN NEONATAL RATS. J.M. Bloom, E.M. Blas and S. Hulse. Dept. of Psychology, Johns Hopkins University, Baltimore, MD 21218.

δ-casomorphins (δ-CMs) represent a group of opioid peptides derived from the enzymatic digests of a milk protein (β-casein). δ-CMs are an interesting class of substances because they produce a means through which mother's milk may affect infants, possibly through an opioid pathway. A number of experiments in adult rats have demonstrated antinociception after central administration of δ-CM.

In the present study we evaluated the antinociceptive effects of δ-CM injected intracerebroventricularly (ICV). In Exp. 2, by centrally injecting naloxone, we evaluated whether the antinociceptive effects of δ-CM were opioid mediated.

In Exp. 1, 10-day-old rat pups were injected ICV either with 25 μg δ-casomorphin-5 (TYR-PRO-PHE-PRO-GLY), 25 μg morphine (for comparison) or isotonic saline, in a volume of 1 μl. Pain responses were recorded at 30, 40 and 60 min. post injection by placing the rats left forepaw on a hotplate (46°C). Paw lift latency (PLL) increased significantly for pups injected with δ-CM and morphine when compared to non-injected pups. Intact pups injected with saline showed a decrease in PLL of 5%. In Exp. 2, pups received an ICV injection of either 25 μg naloxone or isotonic saline, followed immediately by an IP injection of either 0.5 μg/kg δ-CM-5, 1 μg/kg morphine or isotonic saline. Behavioral assessment with naloxone blocked the decrease in heat nociception caused by δ-CM-5 to a level comparable to that of saline.

These results demonstrate that δ-CM can influence antinociceptive behavior in infant rats through a central mechanism that seems to be opioid mediated.

492.10


Recently, it has been reported that diprenorphine substitutes for nalmefene in subjects trained to discriminate nalmefene from distilled water (Geter et al., Neurosci. Abstr., 15:248, 1989), suggesting that there are similarities between the stimulus properties of the two drugs. Given that the two drugs have differential affinities for the various opioid subtypes of the opiate receptor and that animals can differentiate between opiate agonists that differ in this regard, the present study attempted to train animals to discriminate between saline and diprenorphine. Specifically, rats with a history of discriminating nalmefene from distilled water in a conditioned taste aversion drug discrimination assay were given nalmefene prior to a saccharin-LiCl pairing and diprenorphine prior to saccharin alone. Although diprenorphine initially substituted for naloxone, intraperitoneal training subjects acquired the discrimination. In subsequent generalization tests, the substitution was selective with naltrexone serving as a substitute and diprenorphine generalizing to diprenorphine. The subsequent substitution profiles are consistent with their differential receptor activity.

Putative antagonists can be given concurrently with the reinforcer to test for drug discrimination learning (DOL) to assess the ability of the antagonist to block the stimulus properties of the compound. That DOL within the taste aversion design can rapidly acquired and robust may preclude the antagonist. This possible limitation was addressed in the present experiment following the establishment of a morphine discrimination (.5 mg/kg, saline vs 1 mg/kg) was given either 10, 30, 60 or 180 min prior to administration of morphine. When saline preceded morphine administration by 30 and 60 min, it completely antagonized the discriminative effects of morphine. By 60 min, saline no longer antagonized the discriminative effects. That saline blocked the discriminative effects of morphine at these intervals is consistent with other work on the interaction of saline and morphine in more traditional DOL designs and indicates that the rapid acquisition of DOL within the taste aversion procedure does not necessarily preclude its utility in assessing drug antagonism.


Neonatal (3-4 day old) and premature (17-18 day old) Sprague Dawley rat pups were tested following the administration of (0.1) saline, (0.1), 1, and 3 mg/kg MK801. Pups were observed in the best situation 30 and 60 minutes post injection. Behaviors were sampled for 5 seconds every 20 sec. for 5 minutes in each condition. In neonatal rat pups, reductions in a number of behaviors (forward locomotion, mouthing) were seen at the higher (.5 and 3 mg/kg) doses. In contrast, evidence of behavioral stimulation was seen in a lower dose (.1 mg/kg) test for forward locomotion at 30 min. post injection. In premature rat pups, marked sedative effects of MK801 were seen at higher doses (.5 and 3 mg/kg) and with signs of mechanical stimulation increases in forward locomotion and mouthing) evident at low doses. Thus, as in adults, premature pups showed no evidence of behavioral alterations and no dose inhibitory to both neonatal and premature pups, although these effects are somewhat more pronounced in premature than neonatal rats. Investigation of the behavioral consequences of NMDA antagonists such as MK801 is important, given the possible therapeutic potential of these substances for use in young organisms.


Rats were tested in a modified Geller-Selkter (1960) conflict test (MULT FI 60-sec FR 1) to determine the non-competitive NMDA antagonist, 2-amino-4,5-(1,2-cyclohexyl)-7-phosphonooheptanoic acid (NPC 12626), would produce an increase in punished responding during acute and repeated dosing. Chloridiazepoxide (CDP) and phencyclidine (PCP) also were tested.

We found that acute dose presentations, NPC 12626 (30 and 56 mg/kg), CDP (10 mg/kg), and PCP (4 mg/kg) produced selective increases in punished responding. During a six day repeated dosing procedure, the 30 mg/kg dose of NPC 12626 produced a selective increase in punished responding during 5 of the 6 days. CDP (2 mg/kg) produced a selective increase in punished responding on 3 of the 6 days; whereas, the 5 mg/kg dose of CDP produced significant increases in both punished and unpunished responding on 3 of the 6 days. These results demonstrate that the competitive NMDA antagonist NPC 12626 has selective antipunishment effects in an anxiety animal model.

492.14 CHRONIC TREATMENT WITH MK801 PRODUCES TOLERANCE TO THE BEHAVIORAL EFFECTS. L.M. Ford, P.E. Sheehan, S.R. King, and A.B. Norman. Division of Neuroscience, Departments of Psychiatry and Neurology, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Acute systemic injection of MK801, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, increases locomotor hyperactivity, decreased rearing behavior and increased stereotypy (Ford et al., Physiol. and Behav. 46:733-758, 1989). Following chronic treatment with a non-competitive receptor antagonist a tolerance to the behavioral effects is observed. We therefore compared the topography of MK801-induced increases in locomotor activity after acute and chronic treatment to evaluate the possibility of tolerance or sensitization. (n=8) received 0.5 mg/kg i.p. once daily for 21 days; locomotor activity was measured after 1 (acute) and 21st (chronic) injection using the Digiscan Animal Activity Monitor. The increased ambulation (horizontal activity) was not significantly different between acute and chronic MK801 treatments. Locomotor behavior was reduced following acute MK801 treatment compared to saline treated controls (p<0.05). However, following 21 days of treatment these MK801-induced reductions in rearing behavior were reversed and in some cases were elevated above control values. These results indicate that NK1 antagonists may underlie these adaptations in behavior. Supported by University of Cincinnati Research Council, Childrens Hospital (Cincinnati) and NINDS.

492.15 USE OF THE PIGEON CONFLICT PROCEDURE TO CHARACTERIZE ANXIOLYTIC DRUG ACTIVITY: EVALUATION OF N-METHYL-D-ASPARTATE ANTAGONISTS. M. Koest, M.A. Broccos, C.G.A. Randle, and F.C. Colletteaux. Groupe de Recherche du SERVIER, 7 rue Ampère, 92800 Puteaux, FRANCE.

The effects of acute and chronic administration of the clinically effective anxiolytics chlordiazepoxide (CDP), buspirone and ritanserin were evaluated using conflict procedures (Vogel, in Seifter and in pigeons (Barrett). In rats, only CDP was found to have significant antipunishment activity. In pigeons, however, antipunishment effects were produced not only by CDP, but also by buspirone and ritanserin.

Because of its apparent effectiveness in detecting non benzodiazepine anxiolytic agents, the pigeon conflict procedure was used to evaluate possible antiradial activity of various N-methyl-D-aspartate antagonists. punished responding was significantly increased by competitive antagonists (e.g., phencyclidine and MK-801). In preliminary experiments, non-competitive NMDA antagonists thought to act at the NMDA receptor-associated ion channel (e.g., kynurenic acid, 7-chloro-kynurenic acid) failed to show significant antipunishment effects.

492.16 INDOLETHACIN ATTENUATION OF RADIATION-INDUCED HYPERACTIVITY. J.L. Ferguson, S.B. Kandansky, A.H. Harris, M.R. Landager and H.O. Novik, Dept. of Behavioral Science, April, Woods, TX 75485.

Exposure of rats to 5-10 Gy of high-energy electrons produces hyperactivity and reduces motor activity. Previous studies using a GoNoGo source suggest that radiation-induced hyperactivity results from a relatively direct action on the brain and is mediated by prostaglandins. To test this hypothesis, motor activity may be, in part, a thermoregulatory response to this elevation in body temperature, adult male rats were given indomethacin (0.5, 0.5, 1.0, 3.0 mg/kg, i.p.) and either irradiated (LINAC 18 MeV high-energy electrons, 10 Gy at 10 Gy/min, 2.8 usec pulses at 2 Hz) or sham-irradiated. The activity of each animal was measured for 60 min in a photocell monitor for distance travelled and number of vertical movements. Rectal temperatures of irradiated rats given indomethacin were reduced by 0.9-1.1°C at both the beginning and end of the activity session. Indomethacin, at any dose, attenuated radiation-induced hyperactivity without reducing motor activity (Eisenberg and MK-801). The decreased motor activity of the irradiated rats was not affected by any dose of indomethacin. Thus, the increased body temperature following irradiation is probably not a cause for the decreased motor activity.
GENETIC DIFFERENCES IN CONDITIONED TASTE AVERSION. N. E. Collsey and A. C. Collins 1, 2. 1Psychology Department and 2Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

Variability exists in the acquisition of illness-induced consumatory aversions. In an attempt to ascertain if genetic factors contribute to this variability, male mice from 6 inbred strains (A/J/1bg, C3H/21bg, C57BL/6J/1bg, DBA/21/bg, BUB/1Jn, ST/1J) were tested for differences in the propensity to develop conditioned taste aversion. Mice were injected with either nicotine (2 ng/kg) or cyclophosphamide (15 mg/kg) after an ingestion of a novel saccharin solution. Preference testing between water and saccharin was conducted over a 36 day time period. Dose response curves (0-2 mg/kg for nicotine and 0-25 mg/kg for cyclophosphamide) were constructed for 3 of the 6 inbred strains of mice (C3H/21bg, C57BL/6J/1bg, DBA/21/bg). Evidence suggests that there is genetic differences in the acquisition and extinction of conditioned taste aversion and that these differences appear to be dose dependent. Supported by DA-03194 and DA-00116.

DIFFERENTIAL EFFECTS OF A1 AND A2 ADENOSINE RECEPTOR ANTAGONISTS ON PLACE CONDITIONING AND MOTOR ACTIVITY IN RATS. N. T. Brockwell and R. J. Beninger. Dept. Psychology, Queen's University, Kingston, K7L 4N6, Canada.

The recent development of potent antagonists which are relatively selective for A1 and A2 adenosine receptors has made it possible to assess the role of each receptor subtype in modulating behavior. The present study utilized newly developed apparatus which is capable of simultaneously measuring spontaneous motor activity and place conditioning, the most commonly used behavioral measure of drug reward. The experimental design consisted of 3 phases conducted over 12 successive days. During 3 preconditioning sessions, undrugged male Wistar rats received access to two distinctive chambers connected by a small tunnel. During the 8-session conditioning phase, groups were administered either the A1 antagonist CPX or the A2 antagonist CGS 15943 and confined to one of the chambers. On alternate sessions rats were injected with the vehicle and confined to the opposite chamber. On the test session, undrugged animals were again allowed access to both chambers. Results indicate that both 0.1 and 1.0 mg/kg IP CGS 15943 produced significant hyperactivity; 1.0 mg/kg IP CGS 15943 also produced a significant place preference. CPX (0.1 and 1.0 mg/kg IP) failed to significantly alter activity or induce place conditioning. Using an identical procedure, (+)-amphetamine (2.0 mg/kg IP) produced both significant hyperactivity and a place preference as expected. These results suggest the positive behavioral effects previously found with the non-specific adenosine antagonist caffeine (Brockwell, Eikelboom, & Beninger, Neuroscience. Abstract. 1988) may be mediated by the A2 receptor subtype. (Funded by NSERC)

FUNCTIONAL EVALUATION OF SEROTONIN 1C AGONISTS AND ANTAGONISTS BY INTRACELLULAR CALCIUM RELEASE AND PHOSPHONOSTIDIDE HYDROLYSIS IN A CELL LINE EXPRESSING A RECOMBINANT SEROTONIN 1C RECEPTOR. M. Bass, P. A. Hydrop, J. E. Audia, P. Q. Mounsey and L. Yas. Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285; 2Dept. of Medical Genetics, Indiana University School of Medicine, Indianapolis, IN 46223 USA.

AV12 cells were stably transformed with an expression vector, pBD, containing a full-length clone of the mouse serotonin (5HT) 1C receptor. RNA hybridization and ligand binding studies confirmed the expression of the 5HTC1 receptor in the recombinant cell line AIC-19. Stimulation of the AIC-19 cells with 5HT and known 5HT1C agonists resulted in release of intracellular calcium which was detected by dye fluorescence. This response was blocked by known 5HT1C receptor antagonists. The functional coupling of the 5HTC1 receptor to phospholipase C in AIC-19 cells was further demonstrated by increased phosphoinoside hydrolysis in response to 5HT. These studies indicate that the AIC-19 cell line will be useful in evaluating novel 5HT1C receptor agonists and antagonists.


In this study we examined the effect of guanine nucleotides on the binding of the melatonin agonist 2-(15-2)-iodomelatonin (2-IMEL) in P3 membranes and (0.5 μg) of digitonin-solubilized fractions of chicken brain (5 weeks old). These fractions were prepared in 50 mM Tris-HCl buffer (20 μg glycerol, 1 mM mercaptoethanol, 10 mM KCl, pH 7.4). The affinity (Kd) of 6-chloromelatonin for 2-IMEL was lower in solubilized fraction than in membrane fraction. In the membrane fraction (Kd = 214 μM), Guanine nucleotides inhibit 2-IMEL binding in membranes (GTPyS (Kd = 900 μM) < GTP < GDP). However, in the solubilized fraction, GTPyS (0.1 μM) decreased the Bmax from 12.4 to 6.1 fmol/mg protein with no change in the Kd (234 μM) in membrane, but not in the soluble fraction (Kd = 14.5 ± 17 μM, Bmax = 4.1 ± 1.6 fmol/mg protein, n=3). Following reconstitution of a CHO cell line with melatonin receptors, the GTPyS sensitivity of 2-IMEL binding was restored. We conclude that decoupling of melatonin receptors from G-proteins may account for the reduced affinity of 2-IMEL solubilized fractions. Supported by USPHS grants HL 42922 (MEL) and NS 01740 (KCC).
493.3 EBOTIN POTENTIATES ADENOSINE RECEPTOR-MEDIATED INCREASES OF cAMP IN MICROVASCULAR SMOOTH MUSCLE CELLS IN CULTURE. A.A. Bylund, D.B. Bylund, and C.A. Buttrick. Dep. of Pharmacology, Wayne State University School of Medicine, Detroit MI 48201.

Mechanism of cAMP production in vascular smooth muscle cells from rat carotid endothelial resistance vessels. Exposure to 5-ethylcarboxamidoadenosine (NECA, adenosine A2 agonist; EC50 = 2 μM) increased cAMP production a fold in 5-HT (10 μM) cells produced only a small increase in cAMP levels; however, increased (5-248) the maximal cAMP response of NECA and shifted the NECA concentration response curve to the left (2.3-fold). The EC50 for 5-HT was approximately 1 μM. In addition, forskolin (1 μM)-stimulated cAMP levels were also enhanced by 5-HT. The potentiation of NECA-induced cAMP production by 5-HT was antagonized by the presence of the 5-HT antagonist, ketanserin and pizotifen, and mimicked by the selective 5-HT1 antagonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Incubation of the active phorbolester, phorbol 12-myristate-13-acetate (PMA, 1 μM), but not the inactive phorbolester, 4α,12,13-dienophorbole (PDO, 1 μM), enhanced the cAMP response to NECA in a fashion comparable to that of 5-HT. Furthermore, protein kinase C (PKC) activity was increased (approximately 60%) following stimulation with 5-HT for 15 minutes. These data suggest that the potentiation of NECA- and forskolin-induced cAMP production in response to smooth muscle cells by 5-HT is mediated via 5-HT receptor activation of PKC. (Supported by NIH GM-53852, AHA Grant in Aid 890750)


Cultured BON cells of carcinoid tumor origin contain high basal levels of 5HTP, 5HT, and 5HIAA as well as chromogranin, calcitonin, neuropeptide, and preprocalcitonin (PST). PST and FSH are released from BON cells by Ach (blocked by atropine) and by isoproterenol. Both BON cells release PS in vitro upon stimulation with 10^-7 M 5HT. The presence of binding sites for autoreceptors in cultures of cultured BON cells was assessed using [125I]OH-DPAT (8-hydroxy-2-(d-impropylamino)tetralin, selective agonist for 5-HT), binding sites. Scatchard analysis was done using a P2 tissue suspension prepared in Iris buffer (pH 7.4). Data shows significant numbers of 5HT binding sites on BON cells (Bmax: 445 fmol/mg protein) with a Kd of 3.0±0.2 μM. Additional controls included analysis of hippocampal tissue with expected Kd (2.3±0.2 μM) and 445 fmol/mg protein. Taken together, these data suggest that the release of 5HT from BON cells in vitro and carcinoid tumors in vivo may regulate the release of PS and other peptides via 5HT autoreceptors. Supported by NIH HD-220, an grant from Sigma Tau and AG00450.

493.5 DOWN-REGULATION OF THE 5HT2 RECEPTOR IN OPOSSUM KIDNEY (OK) CELL MEMBRANES. P.A. Prieu and D.B. Bylund. Dep. of Pharmacol, Univ of Neb Med Ctr, Omaha, NE, 68110.

We are investigating the regulation of the 5HT2 receptor using membrane binding studies and functional assays. We observe a 40% decrease in 5HT2 receptors in OK cell membranes when cells are pretreated for 18 hr with 10 μM 5HT and in the presence of 5HT (124 pmol) via 5-hydroxytryptamine (124 pmol) as the radioligand. OK cells were plated at 6x10^5 cells per 150 mm culture dish and exposed to 5HT (124 pmol) for 1 hr. Cells were then incubated on day 7 yielded higher Bmax values (+2x). B2 has an effect in these experiments. When cells are exposed to 5HT in the presence (+) of 5HT, Bmax values are not different, however, control - FBS have higher Bmax values than + FBS.

Control - FBS 7 54 ± 4 8 4 ± 2
FBS 7 54 ± 4 8 4 ± 2
FBS 3 40 ± 3 26 ± 2 4

[n] 300, Kp = 0.03, Kp = 0.115; control - FBS + 5HT, FBS + 5HT, FBS + 5HT, FBS + 5HT, Kp = 0.47 (one-tailed paired t test)

[c] 100, n = 3, 2, 2, 2, 2


P11 cells express a high density of 5-HT2 receptors coupled to phosphoinositide (PI) hydrolysis. The effect of exposure to serotonin (5-HT) on the expression and function of 5-HT2 receptors in P11 cells was investigated. In these experiments, PI hydrolysis was measured by the accumulation of 7H-inositol phosphates in the presence of LiCl and drugs. The ability of 5-HT to stimulate PI hydrolysis was greatly reduced or absent following exposure of P11 cells to 10 μM 5-HT for 8 hr. The desensitization of 5-HT mediated PI hydrolysis by 5-HT was time- and concentration-dependent. Exposure of P11 cells to 10 μM 5-HT for 8 hr led to a decrease in the ability of 5-HT to stimulate PI hydrolysis. With longer periods of exposure, concentrations of 5-HT as low as 100 nM caused desensitization of PI hydrolysis. Stimulation of PI hydrolysis by the 5-HT2 antagonist 8-fluoropirenephrine was not affected by prior exposure of cells to 5-HT, suggesting that the desensitization is selective for 5-HT2 mediated PI hydrolysis. The density of 5-HT2 receptors in membranes prepared from P11 cells was measured using 125I-LSD. Exposure of P11 cells to 5-HT resulted in a decrease in the density of 5-HT2 receptors with no change in its affinity for the receptors for 125I-LSD. The decrease in the density of receptors was rapid and pronounced. A decrease in receptor density was observed following exposure to 10 μM 5-HT for 1 hr; after 24 hrs the density of 5-HT2 receptors was approximately 20% of the original value. A decrease in the density of 5-HT2 receptors after exposure of P11 cells to 5-HT may be involved in desensitization of 5-HT2 mediated PI hydrolysis. (Supported by USPHS NS18591).

493.7 GENERATION OF A DNA PROBE FOR THE 5HT7 RECEPTOR IN THE OK CELL, AN OPOSSUM KIDNEY CELL LINE. D.H. Newbury and D.B. Bylund. Department of Pharmacology, University of Nebraska Medical Center, Omaha, NE 68191-8250.

Previous studies from our laboratory (Murphy, T.J., and Bylund, D.B., Mol. Pharmacol., 34:1-7, 1988) have demonstrated the presence of the serotonin 7 (5HT7) receptor in the OK cell line, an established renal proximal tubule epithelial cell line. In order to generate a probe for this receptor, the published sequences of 5HT1, 5HT2 and 5HT3 were used to design degenerate primers directed at the highly conserved sequences in the third and sixth transmembrane domains. OK cell RNA was isolated using an Applied Biosystems nucleic acid extractor. Approximately 40 μg of this RNA was reverse transcribed using Moloney Murine Leukemia Virus reverse transcriptase and AMV reverse transcriptase to cDNA substrates of PCR with the above primers. The resulting product of about 0.7 kb is consistent with the calculated size of this region from the published sequences of other serotonin receptors. The 0.7 kb fragment also hybridizes with a digoxigenin directed towards the fifth transmembrane region of the other published serotonin receptors. This 0.7 kb product is currently being used to screen a lambda gt11 OK cell cDNA library. (Supported by NIH grant GM 07004)


We have recently isolated a DNA sequence encoding a human 5HT3 receptor. This novel serotonin receptor-gene encodes a protein whose amino acid sequence displays relatively low transmembrane homology to other human G protein-coupled receptors: 5HT3 (25%), 5HT3 (25%), dopamine D1 (45%), and eν (45%). Muscle fibroblasts (L6) cells were stably transfected with the DNA encoding this newly derived human receptor gene. Membranes were harvested, and radioligand studies were conducted. Competition studies demonstrated that this receptor interacts with high affinity (Kd= 9.2±0.9 nM) and in a saturable manner (Bmax=4.4±0.1 pmol/mg protein). Competition studies showed a pharmacological profile with rank order of potency: 5CT > 5HTPAMP > 5HTP > 5HT > d-fenfluramine. The coupling of this receptor to G protein(s) was assessed in binding studies using the non-hydrolysable guanine nucleotide analog, Gpp(NH)p. Gpp(NH)p decreased specific [35S]GTPγS binding by 25-50% in a concentration-dependent manner with IC50 = 4.1±1.5 μM. These properties are consistent with the identification of this new clone as a human 5HT3 receptor. The existence of multiple subtypes of 5HT3 receptor has been suggested previously (Xiong and Nelson, Life Sci. 48:1423, 1992). The present clone will provide a useful tool to explore this possibility in the human genome. Further, it has been shown that closely related receptors of the same subfamily (e.g. 5HT3A, 5HT3B, 5HT3C) generally display approximately 75% transmembrane homology, in contrast, receptors from different subfamilies only exhibit approximately 50% transmembrane homology. Therefore, we propose that the cloned human 5HT3 receptor constitutes the first member of a new subfamily of serotonin receptors.

We have recently reported that the cloned human SHT receptor binds both [H]DOB and [H]Mk-801. The SHT receptor binding site represents the agonist high affinity state of the SHT receptor. (Hardt et al, ACNP 1989; Brandkald et al, 2nd Neuropsychopharmacology, Abstracts, 1989). However, when the data obtained in our laboratory displayed a markedly higher proportion of high affinity sites than reported by others and that this ratio could be experimentally manipulated. In order to address possible differences in the saturation expression of multiple distinct sub-types of the SHT receptors, we made use of the high specific activity radioligand [H]DOB (Du Pont-NEN). Specific binding of [H]DOB was displayed high affinity (Kd=0.27nM) and a specific binding (Bmax=124 mol/L). The density of [H]DOB binding sites in these cells was approximately half the total high affinity binding sites seen in the homogenate (1%). The specific binding of [H]DOB inhibitors of high affinity (Ka=200nM) and a specific binding (Bmax=124 mol/L). The density of [H]DOB binding sites in these cells was approximately half the total high affinity binding sites seen in the homogenate (50%).

3.11 MAMMALIAN CELLS TRANSFIGURED WITH A RAT SHT RECEPTOR-CNS: EVIDENCE FOR MULTIPLE STATES AND NOT MULTIPLE SHT RECEPTOR SUB-TYPES. S. Leonard, E. Weisberg, B.J. Hoffman, and M. Teller. Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, N.Y. 12208; Lab. of Neuroendocrinology, NIH, Bethesda, Maryland 20014.

Evidence has accumulated that the SHT receptor exists in different states. The agonist high affinity state is coupled to a G-protein and can be labelled through the use of guanylin-helicagons. [H]DOB and the antagonist [H]-Ketanserin. The agonist low affinity state consists of the free receptor and is labelled by [H]-Ketanserin. Recently, an alternative hypothesis has been put forward proposing that the radioactively labelled binding sites of [H]-Ketanserin and other antagonists radioligands. To show which molecules in this hypothesis is correct, the SHT receptor was transfected into NIH-3T3 (mureine fibroblast) and COS (green monkey kidney) cells. Neither non-transfected cell line expresses the SHT receptor. The transfected cosine cell lines express the high affinity [H]DOB and [H]-Ketanserin, Agonist affinity were significantly higher for [H]-DOB labelled receptors than for [H]-Ketanserin labelled receptors in both cell types. Under the same experimental conditions the ratio of the agonists and antagonists for the binding sites of 1000:1 in mammalian brain homogenates. The [H]DOB binding was guanylin-nucleotide sensitive, indicating coupling to a GTP-binding protein. These data indicate that the SHT receptor gene encodes for the agonist high affinity state and the agonist low affinity state of the SHT receptor. These results strongly support the two state receptor hypothesis and do not support the multiple SHT receptor subtype hypothesis.

4.1 MECHANISMS OF SUPPRESSED ANTIBODY PRODUCTION IN HANDLED MICE. J.D. Dopp, S.G. Schmuck, N. Cohen, and J.A. Mowihlan. Dept. of Microbiology and Immunology, and of Psychiatry, University of Rochester School of Medicine and Dentistry, Rochester, New York. 44622.

Handled mice show behavioral and physiological responses indicative of arousal, with presumed neuroendocrine and sympathetic nervous system inputs. Thus, handling may represent a model of human stress. Mice handled before immunisation show decreased primary IgG antibody levels and matigen-induced T cell proliferation, but unchanged numbers of total spleen cells and percentages of splenic B cells, T cells, and T cells subsets (Mowihlan, et al, Leduc, 1980). To determine which mechanisms that handling suppresses antibody production, we have analyzed some of the immune response to keyhole limpet hemocyanin (KLH).

Group housed female BALB/c mice were held individually 2 days for 2 weeks, while controls remained in their cages. 24 hr later, mice were either sacrificed (Group 1) or injected i.p. with 100 μg KLH (Group 2) and sacrificed 3 or 6 days later. Compared to controls, handled mice in Group 1 had fewer Ig-secreting B cells (CELISA) and decreased levels of spontaneously-produced IgM (ELISA, but unaltered T-cell secretion of Con A induced interleukin-2 (IL-2)). In previous studies, handled mice in Group 2 showed diminished IL-2 induced-proliferation (T~2~)T incorporation and IL-2 stimulated splenic cells.

These results indicate that neuroendocrine and/or sympathetic responses which accompany handling can alter specific immune function as measured by antibody production. Diminished antibody production does not occur by a decrease in IL-2 in the circulation of rats with known to play a role in T cell differentiation. Future studies will focus on levels of T-cell help and on other relevant lymphokines and CNS-derived products that might directly affect immune responses.


The acute stress of surgery is known to suppress natural killer (NK) cell cytotoxicity and increase metastases formation. Using a tumor model of breast cancer in rats, MAB106 adenocarcinoma cells, we recently demonstrated that forced swim stress increases metastases by suppressing NK activity against this tumor. This same malignant cell line, MAB106, synthetic to Fischer 344 rats were used for this study. Thirty-eight rats were divided into 3 groups, subjected to a standard abdominal surgery under halothane anasthesia, to anesthesia only, or to no treatment. Three to five hours after completion of surgery/anesthesia, all groups were injected with 3 × 10^9 MAB106 cells in 5 mL PBS. Twenty-one days later, lungs were taken and surface metastases counted. Surgical stress significantly increased metastatic growth in the surgery group compared to either the anesthesia only or control groups. Taken together with the established role of NK cells in controlling MAB106 metastases, and with our recent evidence that a causal relationship between the effect of swimming stress on NK activity and on metastases, the present finding suggests that surgical stress increases metastases by suppressing NK activity. This hypothesis is now being tested directly in our laboratory. Supported by the UCLA Psychoendocrinology (S.E.B. and R.V.), and NIH grant NS 07628.
491.3


The effects of two opiate drugs, morphine and fentanyl, on metastatic tumor growth were studied using MADB106 mammary adenocarcinoma cell line, previously shown to be controlled by natural killer (NK) cells. Additionally, the cytotoxic activity of NK cells against this tumor, which is synergistic to a murine sarcoma virus, was measured in vitro. Fischer 344 rats were injected either with naloxone (10 mg/kg), or saline, and 20 min later, were injected with either morphine (50 mg/kg), fentanyl (35 μg/kg) or saline. One hour after this administration, the number of intravenously injected with 10⁵ MADB106 cells and two weeks later were removed and surface metastases counted, or sacrificed and NK activity determined in a standard 51 chromium-release assay. Administration of morphine or fentanyl produced a 5-8 fold increase in number of MADB106 metastases in vivo, and markedly decreased NK cytotoxicity in vitro. Naltraxalone completely blocked these effects. In addition, we found that administration of the glucocorticoid antagonist RU38488 (25 mg/kg, one hour before morphine administration) did not attenuate morphine-induced increase in lung metastases, indicating that the effects of morphine are not mediated by glucocorticoids. These findings suggest a causal relationship between the immune suppressive and tumor enhancing effects of opiates. Supported by the UCLA Psychoneuroimmunology Program (R.Y. and S.B.E.) and grants NS 07268 (J.C.L.), AA 06744 and VA Medical Research Service (A.N.T).

491.4


Stress can suppress natural killer (NK) cell activity and increase tumor growth. We have recently provided evidence for a causal relationship between immunosuppressive and tumorigenic effects of stress in an animal model of breast cancer. Using the MADB106 tumor cell line, which is syngeneic to the Fischer 344 rats used in these studies and is known to be controlled by NK cells, we previously showed that stress suppressed NK activity against this tumor in vivo. We now increased the number of metastases in rats inoculated with this tumor. In the present study, rats were either subjected to intermittent forced swimming stress or were not stressed. In the first experiment, with group was injected with the ganglionic blocker, chlorisondamine (3 mg/kg i.p.), or with saline. In the second experiment the animals were either injected i.v. with 2x10⁵ MADB106 cells, and 12 days after surface lung metastases were counted. Stress significantly increased number of metastases in both experiments: chlorisondamine markedly decreased this inhibition, and chlorisondamine completely blocked it. Neither drug affected number of metastases in non-stressed groups. These finding suggest that 9-2 adrenergic receptor activity in the sympathetic nervous system is important in mediating the effects of stress on metastatic growth. Supported by the UCLA Psychoneuroimmunology Program (S. B.-E. and R.Y.) and grants NS 07268 (J.C.L.), AA 06744 and VA Medical Research Service (A.N.T).

494.5


Department of Physical Therapy and thymus, University of South Florida College of Med, Tampa, FL 33613, and Dept of Anatomy*, Texas & A&M Univ., College Station, TX 77843-1114.

Animals exposed to exercise are reported to have normal immune function and viral disease resistance in spite of their severe undernutrition. Possibly the intense exercise commonly performed by these patients helps prevent undernutrition-induced immunodepression. We designed an experiment to test this hypothesis, mice were fed ad libitum or in restricted quantities to induce 25% loss of initial weight over 3 weeks. Half the animals from each dietary group were run on a treadmill for 30 min/day, 5 days/week. Exercise had to effect on several measures of nutritional status. One third of the restricted-exercise mice was removed from the study due to inability to perform the exercise. Antigenic challenge the intestine, spleen weight, thymus and thymus cortex were significantly increased in exercise-exposed mice. In vivo antibody response to sheep red blood cells in vitro spleen responsive to Con A and thyophilaglutaminulin, and production of tumor necrosis factor were not significantly affected by exercise. Serum corticosterone level was increased by food restriction and significantly decreased by exercise in the undernourished mice. These values are being correlated with changes in brain levels of biogenic amines. Regular exercise may help prevent undernutrition-induced immuno-depression by altering the response of animals to stress. Supported in part by NIH (5MH46646) and the Med. Res. Council of Canada.

494.6

THE EFFECTS OF ISOLATION-INDUCED AGGRESSION ON IMMUNE PARAMETERS IN MICE. M.P. O'Grady and N.B. Hallit.

Div. of Psychoimmunology, Dept. of Psychiatry, Univ. of South Florida College of Medicine, Tampa, FL 33613.

Isolation-induced aggression (IAA) was used as a model to assess the effects of a biological manipulation on measures of immunity. In addition, we evaluated the effect of thyomin fraction 5 (TF5), a thymic extract, on modulation of IL-2. TFS stimulates ACTH release which, in turn, has been found to reduce IL-2. Pretreatment with TFS alters neither the number of fights nor the latency to attack. However, the fighters and the Intruders differed in the response of their lymphocytes to mitogens. Compared to Intruders, the fighters were more strongly sensitive to the stimulus. The effect was eliminated by the addition of a thymic extract to the media. Our results suggest that the effects of the isolated environments are primarily the result of the isolation controls (p<0.04). Interestingly, there was a difference between the two groups of fighters as a function of intensity of conflict. TF5 altered the number of fights, but not the number of fights in those animals who fought with a more aggressive Intruder (p<0.006). Thus, thymin weights correlated with these intensity differences in what could be construed as a behavioral "dose-response" curve. Research was supported in part by NS 21210.

494.7


Psychology, University of Pittsburgh, Pittsburgh, PA, 15216.

A variety of stressors can decrease immune function, and the hypothalamic is implicated in this response. In an attempt to define areas of the brain that influence immune function and respond to stressors, c-fos induction was examined following two stress paradigms. Adult male Lewis rats (2-4 g) per group (either) underwent conditioning to foot shock (unconditioned stimulus, US) using auditory clicks as the conditioned stimulus (CS) or were exposed to the CS alone, and 12 days following the conditioning those were either exposed to the CS for 1 hour or were taken directly from their cages. Other animals were either exposed to the US alone or remained naive. Animals were prepared for c-fos staining, as previously described (Hoffman et al., Endocrinology 126: 1736, 1990). C-fos was strongly expressed in cells of the paraventricular nuclei, some of which contain CRF, and other hypothalamic areas directly associated with autonomic function, the septal nuclei, and the orexidial amygdala in animals exposed to a conditioned or unconditioned stimulus. These results might suggest that basal ganglia activation were greatest in the animals exposed to US alone. Control animals exhibited very little c-fos in the diencephalon. C-fos containing brain areas can not be targeted for further study aimed at elucidating their role in stress induced immune suppression.
STRESS-INDUCED SUPPRESSION OF THE CELLULAR IMMUNE RESPONSE TO HERPES SIMPLEX VIRUS (HSV) AND THE EFFECT ON THE ESTABLISHMENT OF ACUTE AND LATENT INFECTION. J.K. Brenchley, M. Fleshner, E.L. Jordan and B. Glaser. Department of Medical Microbiology and Immunology, Division of Medical Sciences, University of Hawaii, Honolulu, Hawaii. It has been well documented that acute or repeated stressful events are able to suppress a broad spectrum of both humoral and cellular immunological responses; however, the effect of stress on the development of specific immune responses has yet to be reported. We have utilized an established murine model of local and systemic Herpes simplex virus type 1 (HSV-1) infection to study the effect of restraint stress on the development of HSV-specific cytotoxic T lymphocytes (CTL) and helper T cells as natural killer (NK) cell activity. These studies were extended to examine the effect of stress on the development and reconstitution of the HSV-specific memory components of the cellular immune response. In addition, the effect of stress on the establishment of acute and latent HSV infection was investigated. Overall, restraint stress was shown to depress the generation of HSV-specific CTL and NK cell activity following local HSV infection at the site of local infection. Restraint stress was also demonstrated to influence the ability to restimulate HSV-specific memory CTL to the lytic phenotype following antigenic challenge. These findings provide evidence that physiological changes associated with restraint stress can influence the ability to generate an immune response to a specific viral infection, thus contributing to the understanding of some of the basic mechanisms underlying stress-associated immune regulation.

INNATE AND ADAPTIVE IMMUNE RESPONSES IN A SOCIAL CONFLICT PARADIGM. M. Lytel, S. Nelson* and N.L. Thompson. 1 Biological Sciences, Mankato State University, Mankato, MN 56002 and 2 Biology, Tufts University School of Medicine, Boston, MA 02111. Innate and adaptive immunity was examined at an in vitro and in vivo level in three strains of mice subjected to social conflict. C57BL/6, DBA/2 and B6AF1 strains were selected on the basis of known differences in neuroendocrine response to chronic daily stress periods when compared to the T-independent antigen PVP was not suppressed. In vitro proliferative responses of spleenocytes to the T cell mitogen Con A and B cell mitogen LPS were unaffected. Acute stress dramatically increased splenocyte phagocytosis while mitogen responses remained unaffected. DBA averaged a 26% increase in phagocytosis as compared to a 41% increase in C57. These findings indicate that alterations in innate immunity in addition to adaptive immunity should also be considered when evaluating neuroendocrine and immune interactions in response to stress and emphasize the importance of using a biologically relevant stressor such as social conflict.

NEONATAL HANDLING AFFECTS IMMUNE FUNCTION AND BEHAVIOR IN PORSOLT'S SWIM TEST. E. Fride, J.A. Hilkoti, and P.K. Aroua. Lab of Neuroscience, NIADDK and Lab of Clinical Studies, NIAAA, National Institutes of Health, Bethesda, MD 20892. A greater resistance to stress and depression has been associated with neonatal immune dysfunction. Friedman indicates that daily handling of neonatal rats results in an increased ability to cope with stress and in delayed development of both ulcers and tumor growth. In the present study, male Sprague-Dawley Wistar rats were handled or not handled from postnatal days 5-20. At 55 days, the time spent immobile in Porsolt's swim test (a putative model of depression) was measured. In 6 months of age, several immune parameters were assessed including natural killer (NK) cell activity and the numbers of splenic B, T, helper/inducer (T) and suppressor/cytotoxic T (CD8+) cells. The latter measures were assessed with fluorescent monoclonal antibodies against cell surface markers for B (mIgG), T (CD3, CD4+ and CD8+), and suppressor/cytotoxic T (CD8+) cells. The duration of immobility the rats exhibited was significantly shorter in handled than in control rats (F(1,136) = 33.5, p < 0.0001). NK cell activity was 40% higher in handled rats (F(1,30) = 12.9). Handled rats had higher immune responses to antigenic challenge than control rats, with a higher CD4/CD8 ratio (p = 0.003). The number of B- and CD4+ cells did not differ between groups. These results suggest that early postnatal handling increased both performance in an animal model of depression and certain aspects of immunocompetence.

BEHAVIORAL, NEUROENDOCRINE AND IMMUNE SYSTEM STUDIES OF HIGH- AND LOW-ADOLESCENT RODENT LINES. E. Mermelblad, N. Castanon, C. Samit*, S. Vichito*, T. Dallmu* M. Le Moal and P.J. Segov* Lab. Psychobiology, Comparative Psychology. INSERM, U7250-IRCA, U1111, Bordeaux II. Domaine de Carreere 33077 Bordeaux Cedex-France. Psychology, Instituto Cajal, Madrid, Spain. Romas strains of Wistar rats have been selected on the basis of their performance in two-way active avoidance behavior and differ also in several other behavioral responses, such as their locomotor activity in a novel environment, the high-adversiveness strain (RHA) being more active than the low-adversiveness strain (RLA). Despite these marked behavioral differences we could not find any between-strain variation in basal levels of corticosterone and ACTH, in their responses to different protocols of novel environment stress, or after CRF challenge. These results suggest that the differences previously described are not necessarily related to the behavioral trait and may be the consequence of a genetic drift or of local environmental conditions. On the other hand the prolactin response to stress was higher in the low-adversive line. The reactivity of spleen lymphocytes was studied in vitro. Natural killer activity against YAC-1 tumoral cells as well as the mitogenic response to concanavalin A were much higher in the low-adversive strain. The genetic link of neuroendocrine variation and immune function differences with behavioral characteristics is currently under study. The available data suggest that the Romas lines of rats are an interesting model for psychoneuroimmunological studies.

IMMUNOSUPPRESSION INDUCED BY A MILD STRESSOR OR STRESSOR-RELATED ODORS. S. Zaleman, L. Ker* and H. Anisman. Dept. of Psychology, Carleton University, Ottawa, Ontario K1S 5B6, Canada. Parallelizing the effects of inescapable foot shock, exposure to a mild stressor (behavioral light stimulus over a 96 min period) applied 72 hr after herpes simplex type 1 inoculation resulted in a marked suppression of the plaque forming cell (PFC) response in CD-1 mice. This treatment also reduced antibody titers, but over the course of several experiments this outcome occurred less reliably. A more pronounced and reliable immunosuppression was noted when the stressor was presented to mice in their home cages. Finally, exposing mice to an environment containing odors of mice that had previously been stressed likewise resulted in an immunosuppression. No such effect was evident if mice were exposed to odors of nonstressed mice. It seems that the PFC and antibody responses are exquisitely sensitive to apparently minor stressors, and raises the possibility that a light stimulus might be inappropriate as a C-S for certain conditions, particularly immunological studies.

1. COMPARTMENT SPECIFIC CELL TRAFFICKING: POTENTIAL MECHANISM FOR STRESS-INDUCED IMMUNOMODULATION. M. Pothier, L. R. Watkins I, L. Lockwood D. Bellera* M. L. 4audenlagier & E. F. Main. Dept. Psych, U CO Boulder, CO 80303. Stressors can alter immune function by many mechanisms. One hypothesis for stress-induced immunomodulation (SI1) is changes in cell populations via lymphocyte traffic. Since immune responses require cooperation of different cell types, stress-induced shifts in cell populations may affect an organism's ability to mount an immune response. We sought to determine if stress could alter lymphocyte trafficking & if so, whether this could be a mechanism for SI1. To test for stress-induced lymphocyte trafficking, male rats either remained undisturbed in their home cages or were exposed to 15 min immobilizing stress. Lymphocytes from peripheral blood, spleen, cervical lymph nodes and superior mesenteric lymph nodes (MLN) were collected and identified as for the cell surface markers: CD3, CD8, IgG, & CD4. Flow cytometry revealed a significantly increased CD4+/CD8+ ratio in MLN of shocked vs. control rats (p<0.001). In contrast, peripheral blood, spleen, & cervical lymph node cell populations were not significantly altered. If this change in cell populations underlies SI1, then immunomodulation should occur only if antigen is processed in the altered MLN. We thus compared the effect of shock on the vivo anti-MLH Ig levels after MLN exposure to KLH (by intraperitoneal injection) or spleen (by intravenous injection). Shock induced reductions in serum IgM & IgG levels (ELISA) were indeed observed. T + (p<0.001) and CD8+ (p<0.005) cells, but not T- (p<0.001) and CD8+ (p<0.002) cells than control rats, resulting in a higher CD4/CD8 ratio (p = 0.003). The number of B- and CD4+ cells did not differ between groups. These results suggest that early postnatal handling increased both performance in an animal model of depression and certain aspects of immunocompetence.

SOCIOLOGY FOR SCIENCE ABSTRACTS, VOLUME 16, 1990

THURSDAY F

Stress can induce changes in immune cell function. The mechanisms(s) for these changes remain unclear. One hypothesis for stress-induced immunomodulation (SH) for which we have evidence (Fleisher, et al., this vol) is changes in lymphocyte populations/immune responses requiring cooperation of different cell types, stress-induced shifts in cell populations render an organism less able to mount effective immune response(s). We use a novel pharmacological modulator of S1 cell trafficking. We focused on 2 systems, the hypothalamic-pituitary-adrenal axis (HPA) and sympathetic outflow. These systems are activated during stress and can influence lymphocyte (lymphoid compartment) and cell trafficking. Specifically, we sought to determine if adrenal medullary hormones, corticosterone or splenic sympathic innervation were critical in cell trafficking. We used either adrenal demedullated (ADM), treated with intracerebroventricular (ICV) dexamethasone (Fleisher, et al. n/a, 1989), or sympathoexposed. Animals other treatments were sham. Blood was collected at baseline and exposed to incescape tilth. Blood samples were collected before (BL) and immediately after (100 hr) stress. ACTH and corticosterone levels were determined (RIA) to verify that 1) the adrenal cortex was still functioning in the ADM rats and 2) ICV Dex blocked HPA activation. Lymphocytes from superior mesenteric lymph nodes (MLN) and cervical lymph nodes were collected and identified as positive for the cell surface markers, CD4 and CD8. BL and 100 hr steroid and ACTH levels in ADM shocked rats did not differ from sham operated shocked rats. Shocked rats treated with ICV Dex had lower 10 hr steroid (not different from BL) and ACTH levels than vehicle treated shocked rats. Flow cytometry revealed a significant increase in CD4*CD8* ratio in MLN in ADM but not in ICV Dex treated rats. Thus, HPA activation but not medullary hormones seems necessary for SH changes in cell trafficking.

94.17 SOMNOGENIC IMPACT OF TRYPANOSOMIASIS IN RABBITS. L. A. Toth-Keller, University of Tennessee, Memphis, TN 38163.

Trypanosomes cause the disease known as sleeping sickness in humans and induce a chronic wasting disease in rabbits. We examined the somnogenic impact of subcutaneous inoculation of rabbits with Trypanosoma. Organisms were first detected in blood 7 days after challenge. Parasitemia correlated to the onset of fever, decreased food intake and elevated fibrinogen levels. The amount of slow wave sleep (SWS) was not markedly altered at this time, but rapid eye movement (REM) sleep was significantly reduced.

On days 7-17 after inoculation, fluctuations in blood parasite numbers and other physiologic parameters were observed. The SWS density gradually decreased below control values, as did delta wave amplitude (DMA) during SWS. The reduction of SWS was first detected during the dark period, but eventually occurred throughout the day. However, a circadian pattern of sleep persisted, as did REMs suppression. These reductions in SWS, DMA and REMs reflect major disruptions in sleep architecture that are likely to be essential to the reduced quality of sleep supported by NS-25378 and NS-26429 from NIH.

94.18 THE SUPPRESSANT EFFECTS OF INTERFERON ON ACTIVITY AND FOOD INTAKE ARE NOT MODULATED BY MEDIATED L. A. Segal & L. S. Crois, Univ Colorado School of Medicine, Denver, CO 80262.

When used to treat cancer, interferon (INF)-a produces fatigue, anorexia, and impaired cognition. A 1600 U/g i.p. injection of mouse INF-a causes a decrease in locomotion and food pellets delivered that lasted 23 hrs after injection (Beh. Neuropharmacol. in Press). One possible mechanism for the decreased activity and consumption is the release of prostanoids induced by INF-a. INF-a was tested by blocking prostaglandin synthesis with indomethacin. DBO/J male mice (n=32) were placed for 2 days in a chamber with a photobeam activity monitor and automated food pellet delivery. Locomotion and number of food pellets delivered were recorded 24 hr/day. On the 2nd day the mice were given one of four i.p. injections: buffer, indomethacin (5 mg/kg), mouse INF-a (1600 U/g), or indomethacin (5 mg/kg) + mouse INF-a (1600 U/g). INF-a significantly suppressed locomotion and food pellets obtained in the 6 hour period after injection, as previously described, however only the effect on food pellet delivery was significant over the 23 hour period. There was no significant indomethacin main effect on any variable, nor INF by indomethacin interaction. Indomethacin did not reverse the effects of INF. The lack of effect of indomethacin suggests that prostaglandins do not mediate the suppressant effects of INF-a on activity and food intake. Supported by MH69718 & MH66621.


Anorexia nervosa is correlated with various abnormalities in activity (1). We are investigating the effects of indomethacin (5 mg/kg) given twice daily on activity and food intake. These cells also bind MDP and produce TNF; both substances are somnogenic. We investigated if prior treatment with INF gamma alters the ability of a hCG-LH fusion to induce a sleep response. INF-a is known to produce TNF in response to INF stimulation. HTB16 cells were grown with or without INF gamma. On the 5th day, INF gamma was removed and the cells were treated with MDP (0.1-1 mg/ml) for 2 days. Expression of HLAa was quantified by ELISA. INF in culture supernatants from cells was measured using an L cell cytotoxicity assay. INF gamma-induced expression of HLA DR in these cells was dose- and time-dependent. MDP (0.01, 0.1 mg/ml) enhanced production of TNF in HLAa+ cells, but not in HLA DR cells. The results suggest that the responsiveness of glial cells to INF depends on prior exposure to INF gamma. We are planning experiments to determine what enhanced TNF production in HTB16 cells is INF gamma dependent. The TNF gene is located within the region of the MHC suggesting that it is involved in the pathology of HLA-associated diseases.

94.20 MURAMYL DIPEPETIDE (MDP)-INDUCED PRODUCTION OF TUMOR NECROSIS FACTOR (TNF) BY HUMAN GLIOBLASTOMA CELLS EXPRESSING HLA a. L. Hon*, A. E. Penttila*, I. Johannsen and J. M. Krueger, Univ. of TN, Memphis, TN 38166.

In humans, the major histocompatibility complex (MHC) class II molecule HLA DR is associated with the narcolepsy (1). Astrocytes express HLA DR in response to IFN-gamma (2). These cells also bind MDP and produce TNF; both substances are somnogenic. We investigated if prior treatment with INF gamma alters the ability of a hCG-LH fusion to induce a sleep response. INF-a is known to produce TNF in response to INF stimulation. HTB16 cells were grown with or without INF gamma. On the 5th day, INF gamma was removed and the cells were treated with MDP (0.01-1 mg/ml) for 2 days. Expression of HLAa was quantified by ELISA. INF in culture supernatants from cells was measured using an L cell cytotoxicity assay. INF gamma-induced expression of HLA DR in these cells was dose- and time-dependent. MDP (0.01, 0.1 mg/ml) enhanced production of TNF in HLAa+ cells, but not in HLA DR+ cells. The results suggest that the responsiveness of glial cells to INF depends on prior exposure to INF gamma. We are planning experiments to determine what enhanced TNF production in HTB16 cells is INF gamma dependent. The TNF gene is located within the region of the MHC suggesting that it is involved in the pathology of HLA-associated diseases.

Supported by Office of Naval Research (N 00014-90-J-0169)

Cyclical release of LH is dependent on the presence of an estrogen stimulus and normal circadian rhythms. While estrogen cyclicity is maintained in intact rats following short term exposure to LL, long term exposure leads to a loss of spontaneous cyclicity. In addition, the opiate system appears to be involved in modulating LH secretion via effects on neurotransmitter activity. Thus, the present study examined the effects of short-term exposure to LL on the LH surge in rats and the role of the opiate system in mediating these effects were examined.

Fisher 344 rats (12:12 LD photoperiod) were OVX and half were placed into LL (Day 0). Rats received a subcutaneous implant of E2 on Day 7 and the right carunum was cannulated on Day 8. On Day 9 rats were bled hourly (1200-2000h). A second group of rats was treated to Day 9 and the third of the rats in LL and LD received NX (10 mg/kg BW) at 0915h, NFX at 1215h or saline at 0915h and were bled hourly (0900-1700h). LH was determined by RIA.

E2 failed to induce an LH surge in OVX rats. An LH surge was evident in LD E2-treated rats by 1500h and peaked between 1700-2000h. NFX at 0915 or 1215h enhanced and advanced the LH surge in LD rats. Also NFX at 0915 elevated basal levels of LH at 1000 and 1100h. NFX treatment in LL rats failed to elicit an LH surge. LL rats responded to NFX treatment with only a brief elevation in basal LH levels.

These data indicate that: (1) the inhibitory effect of LH on the LH surge is enhanced following OVX and (2) the opiate system does not appear involved in mediating the inhibitory effect of LH on the LH surge mechanism.

495.3 *COLOCALIZATION OF ESTROGEN RECEPTOR AND GALANIN IMMUNOREACTIVITY IN THE RAT PREOPTIC AREA. K. L. Carlson and R. E. Watson, Jr.* Department of Neurology, University of Kentucky Medical Center, Lexington, KY 40536-0084.

Galanin (GAL) immunoreactive (ir) neurons are abundant in the preoptic area and hypothalamus of the rat and other species. The present study was conducted to determine if GAL-ir neurons in this species are sexually differentiated components of the preoptic area and that many GAL-ir neurons contain radiolabeled estrogen. This study was designed to conduct if GAL-ir neurons in these sexually differentiated regions express estrogen receptor (ER) immunoreactivity. Inact and gonadectomized female Sprague-Dawley rats were treated with colchicine intracerebroventricularly and perfused with Zamboni's fixative. Frozen sections (20 μm) were incubated in monoclonal antibody to GAL (Abbott Laboratories; 10 μg/ml) and reacted with nickel ammonium sulfamate-diaminobenzidine (DAB) which yielded a blue-black reaction product that was largely restricted to nuclei of GAL-immunoreactive cells. Sections were also incubated in rabbit primary antibody to GAL (Peninsula Labs) and reacted with DAB, producing the typical reddish-brown reaction product in the cytoplasm of immunoreactive cells. Double-labeled cells, characterized by a blue-black (ER) and reddish-brown (GAL-ir) product, were present in the anterior mediolateral premammillary region (Paxinos and Watson, 1986) where GAL-ir neurons are also present. These results lend further support to the notion that GAL-ir neurons are sexually differentiated components of the preoptic area may serve as targets for estrogen action and contribute to expression of hormonally-mediated function. (Supported by the University of Kentucky Medical Center Research Fund.)

495.5 *SYNAPTIC INTERACTIONS OF LHRRH-IMMUNOREACTIVE TERMINALS WITH ESTROGEN RECEPTOR-IMMUNOREACTIVE NEURONS IN THE GUINEA PIG PREOPTIC AREA. M. C. Langub, Jr., B. E. Bailey, and R. E. Watson, Jr.* Dept. Anatomy and Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0004.

During an examination of the relationship of LHRRH and estrogen receptor (ER) immunoreactivity (ir) in the guinea pig brain (Watson, et al., this volume), it was noted that LHRRH-ir neurones were frequently found in close proximity to ER-ir cells. This study was conducted to examine ultrastructurally the relationship of LHRRH-ir terminals and ER-ir cells. Female guinea pigs (Harley strain) were perfused with buffered 4% paraformaldehyde and 0.18% glutaraldehyde and 50 μm brain sections were processed for the sequential localization of ER- and LHRRH-ir. ER-ir was demonstrated with a polyclonal antibody 12232 (Abbott Labs) using TMB as the chromogen (Norenberg and Lehman, 1989). Sections were then incubated in anticasein and reacted using DAB as the chromogen. These two immunoreactive systems were then examined in the same sections to determine whether any coincidence between the two reaction products was present. In the present study, LHRRH-ir and ER-ir cells were present in the internal precerebral area, anterior hypothalamus, area of the tuber cinereum, infundibular nucl., and ventral nucl. (nomenclature from the atlas of Bleris, 1983). However, in the 8 analyzed brains, only a few LHRRH cells have been found to show also an immunoreactive to ER. Frequently, LHRRH-ir neurons were located in immediate proximity to many ER-ir cells, which were often arranged in clumping or aggregates. Interestingly, at the most anterior aspect of the preoptic area, abundant varicose LHRRH-ir fibers streamed directly past many ER-ir cells suggesting the possibility of syinaptic interactions between the two (see Langub, et al., this volume). These results indicate that in the guinea pig the preoptic region of the LHRRH system is innervated by estrogen receptor positive neurons. (Supported by the University of Kentucky Medical Center Research Fund.)

495.6 *EFFECT OF GONADOCYTOR ON GNRH CELLS IN THE NERVOUS TERMINALS AND PREOPTIC AREA IN MALE AFRICAN CICLIDHS, HAPLOCHROMIS BURTONI. R. G. Evans, R. D. Fernald* and B. J. Zamboni Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

Magnocellular neurons in the preoptic area (POA) that are immunoreactive to GNRH control the rate of GnRH secretion in males of Haplochromis burtoni. The size of these neurons is influenced by the social environment, such that dominant individuals exhibit large soma sizes and subordinate individuals relatively small soma sizes. Here I sought to determine whether androgen feedback is required to maintain the large cells in dominant fish. Subjects were either gonadectomized or sham-operated. After four weeks all fish were sacrificed. Gonadectomized fish were examined for residual testes. Their brains were then removed, sectioned on a cryostat and stained with an antibody to salmon GNRH. Gonadectomized fish showed no reduction in soma size relative to controls, nor any difference in the intensity of the antibody stain. In addition, there was no reduction in aggression in gonadectomized males compared to sham-operated fish. These results suggest that circulating androgens do not modulate soma size in GnRH-secreting neurons of adult males, and that aggressiveness too, is relatively independent of androgen levels.
**HYPOTHALAMIC-PITUITARY-CONTRIBUTIONS TO STEROIDS AND MONOAMINES**

**5. GABA RECEPTOR ANTAGONISTS INCREASE LHRLH NEURONAL RESPONSIVENESS TO NOREPINEPHRINE (NE)**

GABA antagonists increased LHRLH neuronal responsiveness to norepinephrine (NE). Electron microscope studies revealed that NE caused a significant release of LHRLH into the synaptic cleft, generally within 5 min after NE injection. The facilitated release was associated with a decrease in cell somatic area and a slight increase in dendritic length. These results suggest that NE may modulate LHRLH release by acting at presynaptic GABA receptors on the hypothalamic neurons that synthesize LHRLH.

**6. LUTEINIZING HORMONE (LH) PULSE FREQUENCY, BUT NOT AMPLITUDE ARE REDUCED FOLLOWING INHIBITION OF DOPAMINE-PERKSONSYLYASE (DBH)**

DBH inhibition reduced LH pulse frequency but not amplitude. These results suggest that DBH inhibition may play a role in the regulation of LH pulse frequency, possibly through modulation of dopamine levels. Further studies are needed to elucidate the mechanisms underlying these findings.

**7. ULTRASONIC VIBRATION AND LHRLH FROM THE SAME ANTERIOR PITUITARY PULSES IN FEMALE RATS. SAMPLE INTACT AND CASTRATED MALE RATS.**

Ultrasonic vibration increased LHRLH release from the anterior pituitary of female rats, but did not affect LHRLH release from castrated male rats. These findings suggest a sex-dependent mechanism for the effects of ultrasonic vibration on LHRLH release, possibly involving different regulatory pathways in males and females.

**8. DISTRIBUTION OF ESTROGEN RECEPTOR (ER) CONTAINING AND GABAA RECEPTORS IN THE RAT MAMMARY GLAND:**

This study investigated the distribution of ER-containing and GABAA receptor subtypes in the rat mammary gland, using immunohistochemical techniques. ER-containing and GABAA receptor subtypes were found to be colocalized in the glandular epithelium and stroma, indicating a possible role for these receptors in the regulation of mammary gland function.

**9. OVARIAN HORMONES INCREASE DOPAMINE RECEPTOR CONCENTRATION IN THE HYPOTHALAMUS AND STRIUM OF THE RAT.**

The administration of ovarian hormones increased dopamine receptor concentration in the hypothalamus and striatum of the rat, suggesting a role for these hormones in the regulation of dopamine receptor expression in these brain regions.

**10. BENZODIAZEPINE-INDUCED INHIBITION OF RAPIDLY ALTERING NEURONAL TRANSITION BETWEEN THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS:**

Benzodiazepines were shown to inhibit the rapid transition between the hypothalamic-pituitary-adrenal axis, possibly through a mechanism involving the modulation of neuronal excitability and neurotransmitter release. Further studies are needed to elucidate the specific mechanisms underlying these effects.
To HYPOTHALAMIC K, received also adrenergic pulses (NAL), (p<.05).

LH-releasing level increased gradually from 0900 in excitatory doses of P01-HD21921 to 1000.

PRL levels were also increased 22% by chronic E2 and then rebounded 33% to 10 post-E2, whereas 1500 levels were increased only after 20 d.

At 10 post-E2, cytoplasmic and nuclear POMC mRNA levels were greater at 0900 than at 1500 h.

Schmidt, Kerdelhue*, Lederger*, and Levine, Neurobiology of Aging, University, Evanston, IL 60208.

The present study was designed to assess the simultaneous changes of turnover of noradrenaline and adrenaline in the preoptic area in relation to the luteinizing hormone releasing hormone (LHRH) cell bodies and in the median eminence, the site of the LHRH terminals. Rats were maintained with light on from 06.00 to 20.00. The turnover rates were measured every 10 min after pro-oestrous, and 12.00, 15.00 or 17.00. E2-induced increase in pro-oestrous was lost on chronic oestrous injection of 20 mg E2.

These results, histologically verified samples suggest that the occurrence of the LH surge is associated with increased adrenergic turnover in the region of the LHRH-containing perikarya and terminals.

HYPOTHALAMIC-PITUITARY-GONADAL MODULATION: REGULATION BY STEROIDS AND MONOMERS.

SIMULTANEOUS ESTIMATION OF ADRENALENAL AND NORADRENALINE LEVELS IN THE PREOPTIC AREA AND MEDIAN EMINENCE OF PRO-OESTROUS AND DIOESTROUS RATS. J. Opalka-Jeffry* and C.W. Coen, Division of Biomedical Sciences, King's College London.

The exocytotic acid amon rh-N-Methyl-D-aspartate (NMDA) stimulation in lh-releasing hormone (LHRH) secretion. These studies were conducted after administration of LH secretion by injections of NMDA to the rats, (ii) if any NMDA effects on FSH are mediated by LH, and (iii) if LH and FSH responses may change after the endogenous status.

Castrates and sham-castrates received 1 mg/kg ip every 10 min for 4 h. Plasma LH and LH secretory rate in all groups (control vs. vehicle vs. NMDA) were increased by 100% (p<.01), while FSH levels were unchanged following administration of NMDA. LH responses to NMDA were measured in 21 male rats compared to sham-castrates. FSH levels were also unchanged following NMDA in castrates.

The present study demonstrates that LH surge is associated with increased adrenergic turnover in the region of the LHRH-containing perikarya and terminals.

Because adrenergic neurons contain a high affinity for NAL, it was used in a model in which rat mediodorsal hypotalamic (mHVT) dopaminergic, but apparently not noradrenergic, activity has been demonstrated. To determine at high adrenergic (E2) and then withdrawn in the weeks following removal of the E2, surpassing pre-suppression levels. Under at least steroidal conditions MBH dopaminergic activity is higher in the mHVT and the caudal hypothalamus, we also added 100 and 1000 HVT rats were ovariectomized (OVX), OVX with 3 d (18 pg/ml), 0 h, or with 20 h or 90 pg/ml E2, or OVX with 20 h or 10 d, 20 h after E2 injection, received 10 mg/kg ip of NMDA or vehicle.

The nature and mechanism of adrenergic (E2) effects on tuberoinfundibular dopaminergic (TIDA) neurons are still controversial. It has been proposed that E2 modulation (in parallel) on the activity of tyrosine hydroxylase (TH) in the median eminence (ME) and on serum prolactin (PRL) release. Twelve days ovariectomized rats were injected with I(20)H (20 pg) and sacrificed hourly from 0.00 to 10.00. TH activity was quantified by measuring the amount of enigmatic tyrosine converted to L-DOPA f.e.u. and measured by the estradiol-induced PRL elevation. To interpret the present study we also decreased the E2-induced decrease in TH activity. Another study was performed to study the kinetics of TH in the ME as well as its quantity (by Western blot analysis). The decrease in TH activity after E2 treatment nicely paralleled an immediate decrease in the affinity of its cofactor (E2-PH), while E2 was unchanged. A decrease in the amount of TH was also observed but its latency preceded its major involvement in the immediate decrease in TH activity. Therefore, when observed independently from those of PRL, E2 effects on TH in TIDA neurons are clearly inhibitory. They consist in a desensitization of the enzyme together with a reduction of its synthesis.
HYPOTHALAMIC-PITUITARY-GONADAL REGULATION: MODULATION BY STEROIDS AND MONOAMINES

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

08.19 COMPARISON OF LH AND PROLACTIN RESPONSES TO KAINATE AND NMA IN LACTATING AND CYCLING RATS. R. Abbot* and R.S. Smith, Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

We have reported that in cycling rats, NMA (20-40 mg/kg ip) stimulates both LH and prolactin (PRL) release (Endocrinology 124: 1905, 1989). Similar results were obtained in lactating rats, but this effect was not observed in cycling rats. In the present study, both LH and PRL responses to NMA were observed in cycling rats, but only LH was increased in lactating rats. This suggests that there may be a differential effect of NMA on LH and PRL release in lactating and cycling rats.

08.20 THERMOGENESIS IN VIVO RELATING TO STIMULATION OF NEUROBIOLOGICAL CORRELATES OF STIMULATION. J.A. Thornehill and I. Halvorson, Dept. of Physiology, Unives. of Saskatchewan, Sask., Canada S7N 0W0.

Thermoregulatory experiments were conducted in conscious and urethane anesthetized male Sprague-Dawley rats previously acclimated for 3 weeks to 4°C or 23°C to determine if VPVm electrical stimulation or intravenous infusion of saline or porcine VPVm could stimulate thermogenesis in rats. VPVm injection was significantly increased in VMH temperatures (TPvm) above core temperature when anesthesia was maintained (1.5 g/kg ip). TPvm increased by approximately 1°C when anesthesia was maintained. When anesthesia was maintained, the increase was approximately 0.5°C. These findings suggest that VPVm injection in the VMH region may be involved in the regulation of thermogenesis. It is possible that VPVm injection in the VMH region may be involved in the regulation of thermogenesis.

08.21 REGULATION OF AUTONOMIC FUNCTION: TEMPERATURE REGULATION AND NEURONAL IMMUNE SYSTEM INTERACTIONS.

THURSDAY PM

496.1 BROWN ADIPOSE TISSUE RESPONSES OF CONSCIOUS AND ANESTHETIZED RATS FOLLOWING VMH ELECTRICAL OR INTRAVENOUS KAINATE STIMULATION. J.A. Thornehill and I. Halvorson, Dept. of Physiology, Unives. of Saskatchewan, Sask., Canada S7N 0W0.

Thermoregulatory experiments were conducted in conscious and urethane anesthetized male Sprague-Dawley rats previously acclimated for 3 weeks to 4°C or 23°C to determine if VPVm electrical stimulation or intravenous infusion of saline or porcine VPVm could stimulate thermogenesis in rats. VPVm injection was significantly increased in VMH temperatures (TPvm) above core temperature when anesthesia was maintained (1.5 g/kg ip). TPvm increased by approximately 1°C when anesthesia was maintained. When anesthesia was maintained, the increase was approximately 0.5°C. These findings suggest that VPVm injection in the VMH region may be involved in the regulation of thermogenesis. It is possible that VPVm injection in the VMH region may be involved in the regulation of thermogenesis. It is possible that VPVm injection in the VMH region may be involved in the regulation of thermogenesis.

496.2 VENTROMEDIAL HYPOTHALAMIC SIMULATION OF BROWN ADIPOSE TISSUE (BAT) THERMOGENESIS. W. P. Nakagawa, K. H. Kelloff, and J. B. Hennessey, Dept. of Physiology, University of Ottawa, Ottawa, Canada, KIN 6N5.

The ventromedial hypothalamic nucleus (VMH) is innervated by the VMH region. This area is known to be involved in the regulation of thermogenesis, and is known to inhibit the release of endogenous opioids. However, the exact mechanism by which the VMH region inhibits thermogenesis is not known. The present study was designed to investigate the role of the VMH region in the regulation of thermogenesis. The VMH region was stimulated by the injection of VMH neurons, and the effect on thermogenesis was measured. The results of this study suggest that the VMH region is involved in the regulation of thermogenesis, and that the VMH region is responsible for the inhibition of thermogenesis.


SHRs exhibit diminished ability to maintain body temperature in the cold, but the pathophysiological mechanism responsible for this deficiency is unknown. We studied the possibility that failure of SHR to maintain body temperature in the cold is related to a reduced ability to centrally activate adrenergic thermogenesis in BAT. In the present study, injection of PGF2alpha (20-300ng) into the POAH had a differential effect on intracerebroventricular (ICB) of adult, urethane-anesthetized SHR and nonanesthetized WKY. The effect of PGF2alpha on the core temperature of rats was later evaluated.

496.4 THE INFLUENCE OF ONE-KIDNEY RENAL VASCULAR HYPERTENSION ON THE HYPERTHERMIC RESPONSE TO CENTRAL PGF2alpha IN THE RAT. D.M. Pils, D.E. White, and O.J. Fitzpatrick, Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

There is evidence for high circulating levels of arginine vasopressin (AVP) during development of one-kidney/one-clip (1K1C) hypertension. Arginine vasopressin is also known to act centrally as an rodent and anti-vasopressin. However, the exact mechanism by which AVP affects blood pressure is not known. The present study was designed to investigate the role of AVP in the regulation of blood pressure in rats with 1K1C hypertension. The results of this study suggest that AVP is involved in the regulation of blood pressure in rats with 1K1C hypertension, and that AVP is responsible for the increase in blood pressure observed in these rats. The results of this study suggest that AVP is involved in the regulation of blood pressure in rats with 1K1C hypertension, and that AVP is responsible for the increase in blood pressure observed in these rats.

In recent years, the role of prostaglandin (PG) in the brain has been studied extensively, but the exact functional sites of PG in vivo have not been determined. Therefore, we performed autoradiography of 3H-PGE2 binding sites in the AV3V region. The high density of 3H-PGE2 binding sites was observed in the AV3V region. The functional sites of PG in vivo might be in the AV3V region.

496.6  SENSITIVITY OF RAT MEDIAN PREOPTIC NEURONS TO TEMPERATURE, ANGIOTENSIN II AND NORADRENALINE. E.A. Travis and A.K. Johnson, Deps. of Psychology and Pharmacology and the Behavioral and Social Sciences, Michigan State University, East Lansing, MI 48824.

The median preoptic nucleus (MPO) receives angiotensinergic projections from the subfornical organ and noradrenergic projections from the lateral tegmental and periaqueductal gray. ANG II and NE potentiated the pyrogenic response of MPO neurons in vitro. In vitro, ANG II (10^{-6} - 10^{-5} M) and NE (10^{-6} - 10^{-5} M) increased the membrane potential of MPO neurons in vitro. In vivo, ANG II and NE potentiated the pyrogenic response of MPO neurons in vivo.

496.7  ALTERED BRAIN [3H]DIAZOXON BINDING IN RABBITS DURING FEVER. L.K. Vaugum, Marquette Univ. Sch. of Dentistry, Milwaukee, WI 53201.

The mechanisms responsible for fever are incompletely understood and the possible role of changes in the number and/or affinity of brain receptors for thermoregulatory neurotransmitters during fever is unknown. We investigated whether fever is associated with changes in opioid receptors (\alpha-2 AR) since porcine PGE2 injected into the hypothalamus is known to act as a neurotransmitter in fever. Our results indicate that fever may not be associated with changes in the number and/or affinity of brain receptors for thermoregulatory neurotransmitters during fever.


The neuropeptide arginine vasopressin (AVP) has been shown to act within the central core area of the brain in conscious animals as an endogenous antipyretic. AVP exerts a pyrogenic effect on the spinal cord (1) and increases mean arterial pressure (2). The authors determined whether AVP is involved in the mediation of the pyrogenic effect of prostaglandin E2 (PGE2) on the central core area of the brain in the urethane-anaesthetized rat.

496.9  THERMOREGULATORY CONSEQUENCES OF HIPPOCAMPAL TRANSECTIONS AND SEPTAL LESIONS IN HAMSTERS: DELAYED RESPONSES TO EXOGENOUS PYROGEN AND DELAYED DROP IN BODY TEMPERATURE. E. Johnson and K.L. Borg. Dept. Kinesiology, Unv. of Michigan, Ann Arbor, MI 48109.

The current study was designed to determine the thermoregulatory consequences of rostromedial septal lesions (SEP) and hippocampal transections (HIPP). Male, 4- to 6-week-old, hooded, Sprague-Dawley rats were used. The results indicate that HIPP induces a delayed drop in body temperature and that SEP reduces the magnitude of the thermoregulatory response to exogenous pyrogen.

496.10  EFFECT OF INTERLEUKIN-1\alpha ON RADIATION-INDUCED HYPERThERMIA IN Rats. S.B. Kandasamy, K.S. Kumar, A. A. Harris, and J. F. Weiss. Behavioral Sciences and Radiation Biochemistry Departments, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

A previous study demonstrated that IL-1\alpha increased the level of prostanoids and decreased the level of prostaglandin E2 (PGE2) in rats. The current study determined the effects of IL-1\alpha on the radiation-induced hyperthermia response in rats.

SOCIETY FOR NEUROSCIENCE Abstracts, Volume 16, 1990

THURSDAY PM
496.11 RESPONSES OF PREOPTIC NEURONS TO HUMAN RECOMBINANT INTERLEUKIN-1 (IL-1) IN TISSUE SLICES. L. Xin* and C.M. Blatteis. Univ. of Tennessee, Memphis, TN 38163.

It has been suggested that IL-6 may be another endogenous pyrogen (EF) because, like other such agents, its i.v. injection induces monophasic fevers, albeit at much higher doses than the others. We recently showed that site of action in guinea pigs in the preoptic area (PO); this is also the locus of the pyrogenic action of the other EFs. This brain region contains cold- (C) and warm- (W) sensitive neurons that generally are excited and depressed, respectively, during the febrile rise of other EFs. The chemically induced activities of warm-sensitive neurons were recorded in 200-μm-thick slices of free nerve endings (FNE) and thermosensitive neurons in response to IL-1β in rats were studied. IL-6 is affected by IL-1β and other EFs in a dose-dependent manner. The results indicate that IL-1β is involved in the thermosensitive pathway in the PO.

496.12 FOOD INTAKE AND BODY TEMPERATURE RESPONSES OF RATS TO RECOMBINANT HUMAN INTERLEUKIN-1β (IL-1β) AND IL-1β ANTAGONIST (IL-1β ANTIG). C.L. McLaughlin, G.J. Rogan and C.A. Bailey. Animal Sciences Division, Monsanto Company, St. Louis, MO 63108.

The cytokine IL-1β, produced by activated macrophages, may mediate the cachexia associated with cancer. Food intake (FI) is decreased by acute but not chronic administration of IL-1β to rats. IL-1β also induces analgesia and this effect was blocked by Lyso-1-Pro-Thr, an IL-1β antagonist (Perrier, S.H., et al., Nature 334:698, 1988). In the present experiment the ability of this IL-1β antagonist block the decreases in FI and in body temperature (BT) responses to IL-1β were tested in male Sprague-Dawley rats. IP administration of 1.25, 1.88 and 3.5 μg of IL-1β decreased FI, 20 and 19% and 2-h after treatment (25.2, 21.9 and 20.2 °C, respectively, p<.05). Cumulative 22-h FI was decreased for the 2 higher doses only. In experiment two the decrease, FI in 4-h after 1.25 μg IL-1β (3.0 and 12.5 μg, respectively, p<.05). Cumulative 22-h FI was decreased similarly by 1.25 μg IL-1β alone and in combination with 1 or 5 mg IL-1β antagonist (25.0, 25.6 and 26.0 ± 3.2 °C, respectively). The IL-1β antagonist alone (3.0 and 10.0 mg/rat) did not affect cumulative 22-h FI (31.3, 10.5, 25.3 and 31.0, respectively). Increased BT 4 h after 1.25 μg/rat IL-1β (9.7 vs control. -1. C, p<.05) was blocked by 5 and 25 mg IL-1β (1.2 and 2.4, respectively). NS. BT 22 hrs later was not affected. It is concluded that IL-1β antagonist can block FI and BT responses to IL-1β in addition to the analgesic responses demonstrated previously.

496.13 DISTRIBUTION OF CYCLOOXYGENASE (PGH2 SYNHATASE)-LIKE IMMUNOREACTIVITY IN THE HUMAN CEREBRAL NEURVOS SYSTEM. C.D. Bredere and C.S. Shapiro. Dept. of Pharm. & Physiol. Sci. and Neurology, Univ. of Chicago, Chicago IL 60637

Cyclooxygenase (COX) is the rate limiting enzyme in the conversion of arachidonic acid to prostaglandins. These lipid mediators have been implicated in numerous central nervous system activities including modulation of the febrile response, modulation of peptide neurotransmission in the cerebral cortex and depolarization of sensory afferent neurons. We mapped in detail the distribution of COX-like immunoreactive (ir) structures in the ovine central nervous system with two polyclonal antisera raised against the purified enzyme. COX-ir neurons were observed throughout the cerebral cortex that varied in different areas. COX-ir neurons were particularly numerous in the basal forebrain, particularly in the tuberomammillary and dorsomedial hypothalamic nuclei, the basal amygdala and the bed nucleus of the stria terminalis. In the brainstem they were found in somatic sensory cell groups including the dorsal column nuclei and the spinal trigeminal nucleus as well as visceral sensory regions such as the nucleus of the solitary tract and parabrachial nucleus. COX-ir non-neuronal structures were observed in association with the circumventricular organs of the third and fourth ventricle. We propose that COX may be present within the CNS particularly in neuronal pathways involved in sensory and autonomic function.


These exist a close correlation between blood pressure and heart rate during recovery from fever (F) and blood pressure by endotoxin fever. AVP released into the VSA, reduces body temperature during fever. Blood pressure responses to three different substances have been located in the VSA, therefore neuronal connections within the VSA may play an important role in modulation of body temperature by blood pressure during fever. A rat. Telemetry was used to measure MAP and HR from unestra tified rats females. Blood pressure measurements revealed the following: MAP and HR were both lower than normal. Rats were treated with injections of endotoxin every 3 days. The VSA was infused for the length of the experiment with 10% lidoaline or vehicle (1 ml/hr). Linear regression analysis of vehicle-treated tolerant rats showed no relationship between Tb and MAP whereas lidoaline-infused tolerant rats had a good correlation (r=0.74). These results lead us to conclude that the VSA mediates some of the pathways between Tb and MAP in the tolerant rat.

497.1 PRENATAL EXPOSURE TO DIETHYLTILBETROLER AND CHANGES IN THE IMMUNE SYSTEM. N.T.S. Hall and M.P. O'Grady. Psychoneuroimmunology Div., Dept. of Psychiatry, Univ. of South Florida College of Med., Tampa, FL 33613.

We have previously shown that thymosin peptides can modulate reproductive neuroendocrine circuits and that activation of the immune system during ontogeny can modulate the adult reproductive axis. In this study, we evaluated the effects of early exposure to a synthetic neuroendocrine compound, diethylstilbestrol (DES). Pregnant SW mice were injected with 1 μg of DES either between days 11-15 or days 17-20 of gestation. Control mice received the same volume of oil vehicle or remained un.injected. Overall, early exposure to DES was found to enhance mixed lymphocyte responsiveness, and blastogenesis in response to Con-A, PMA and LPS in the 4-5 week old male and female offspring. Control males had increased weight and spleen cell blastogenic responsiveness compared with control males while DES exposure increased the male measures toward those of the untreated female offspring. These results suggest that exposure to embryonic compounds during fetal development can alter the immunologic status of the adult. Supported in part by a grant from the NIH (NS21210).

497.2 IMMUNOLOGY DISPARITY IN THE WEEP/D2 LITTER MICE. T.J. Crooks, L.J. Borensky and E. Remmers. College of Medicine, University of Kentucky, Lexington, KY 40536-0084.

The D2/JW dwarf mouse, a homozygous recessive mutation lacks growth hormone (GH) and prolanl and can be characterized by extremely stunted growth. Reports by several laboratories indicate that these mice are also compromised immunologically implying a role for neuroendocrine hormones in the development thymus and spleen and are deficient in both humoral and cellular-mediated immunity. These immunologic abnormalities can be corrected by supplemental treatment with GH and thymosin or GH alone. However, more recent data show that these mice are not impaired immunologically and have a normal percentage of T-cell and B-cells in the spleen and cell-mediated immunity to oxazoline, a skin sensitizing agent. This laboratory indicates an immunological disparity exists in this strain of mice. Although all mice are small in size, some have normal proportions of T, B-cells and T-cell subsets in the spleen, while others show an abnormal predominance of T-cells over B-cells. The thymus is also not impaired in that the population of CD4+, mature T-helper cells predominate with very few immature CD4/CD8 cells, the cell type normally found in highest frequency. (Supported in part by USPHS grant NS25225).
497.4


Two we harvested and examined the muscarinic acetylcholine receptors that have been identified in the adult mouse thymus. However, specific CHAT has only been identified within tissues in one mouse strain, C57BL/6, in immunocytochemistry. In this study, we have examined the thymuses and brains of fourteen adult male and female B6/c mice, five to six weeks of age for specific CHAT activity as described by Badanchian and Carroll. J. Neurochem., 5:1955, 1985. Our results show that CHAT activity for whole brain was 9.9 +/-.07 µmol/min/mg protein and 2.51 +/-.07 µmol/min/mg protein for the thymus. The levels of CHAT activity would be expected for tissue such as the brain (which includes both cholinergic neuronal cell bodies and fibres) than for thymus (which contains only cholinergic neuronal fibres). Future studies will determine if the activity of CHAT in the thymus is influenced by development, circadian rhythms or hormonal stimulation. Supported by SRR grant N00014-89-J-1256.

497.5

LUTENIZING HORMONE-RELEASE HORMONE GENE EXPRESSION IN THE THYMUS. B. Marchetti, C.C. Maier* R.D. LeBeau*, and J.B. Bialeck*. Dept. of Pharmacology, Univ. of California, Davis, Calif. and Dept. of Physiology and Biophysics, Univ. of Alabama at Birmingham, Birmingham, 35294 U.S.A.

The luteinizing hormone-releasing hormone (LHRH) gene is expressed in hypothalamic and extra-hypothalamic brain regions, as well as in mammary gland, gonada, and placenta. These results suggest that LHRH plays an important function role in the control of reproductive functions. Recent evidence (Marchetti, B. et al., Endocrinology 125:1025, 1989) indicates that LHRH is able to directly influence the expression of its receptor both in vivo and in vitro via specific LHRH receptors. In this report, we show that LHRH is expressed by immune cells. Poly A+ cDNA from thymocyte and spleen cDNA preparations was used as a template for selective RNA-P or PCR products. Amplification primers were designed to yield a 330 bp cDNA product. PCR products were sequenced and compared to the sequence of hypothalamic LHRH. The results of this study provide the first characterization at the molecular level of LHRH gene expression by cells of the immune system and identifies another potential regulatory circuit between the neuro-endocrine and immune systems.

497.6

ACCELERATED DEVELOPMENT OF MONOAAMINERGIC INNERVATION OF KIDNEY, SPLEEN AND THYMYUS IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). Vincent M. Canouse II and Edwin S. Purdy*. Dept. of Anatomy and Cell Biology, The University of Kansas Medical Center, Kansas City, Kansas.

The SHR has several pathologic traits which accompany the elevated blood pressure, including increased activity of renal sympathetic nerves, renal damage and immune deficiency compared to the normotensive Wistar Kyoto rat (WKY). The augmented sympathetic nerve activity is thought to contribute to the pathogenesis of hypertension as well as the vascular abnormalities in SHR. We propose that augmented or early sympathetic innervation might contribute to renal changes and immune deficiency in the SHR. To test this hypothesis, the sympathetic nerves to the kidney, spleen and thymus of SHR and WKY rats (newborn, 1, 2, 3 and 12 weeks of age) were examined using the glyoxylic acid histofluorescence method. Evidence was used in support of the hypothesis that sympathetic innervation is increased in SHR all three organs. The kidney and thymus exhibited a sustained increase in innervation while the spleen exhibited only a transient increase. In conclusion, kidney, thymus and spleen are innervated precociously in the SHR as compared to the WKY. Renal innervation may predispose the kidney to hypertensive damage while thymic and splenic innervation may contribute to the immune deficiency of the SHR. Supported by PHS grant #M01RR00045.

497.7


We have compared the extent of adrenal steroid (A) receptor (AR) level and down-regulation that occurs between different tissues in response to removal of endogenous adrenal steroids (via adrenalectomy, A) and replacement with exogenous corticosterone (CORT; via subcutaneous pellets). In rat and mouse, A (30 mg) were given for 5 days. Plasma CORT pellets ranging from low- to high physiological values. All CORT pellets were removed 24 h before sacrifice and RIA of trunk blood from each individual animal indicated that there was no residual CORT at the time of sacrifice. After sacrifice, the AR level in the cortex (AR/CORT; A/CORT group) was used to the reference for the number of AR receptor present in the non-up or down-regulated state. The type I AR receptor (AR-I) is not up-regulated with in 6 days of A/CORT, but was down-regulated significantly in the hippocampus by both the adrenals and the high dose CORT replacement, and in the spleen by the high dose CORT. The type II AR receptor (AR-II) is up-regulated in the hippocampus by both the adrenals and the high dose CORT, but was down-regulated in a variety of brain areas (hippocampus, cortex, the pituitary, and the thymus) in all immune tissues (thymus, spleen, and peripheral blood mononuclear cells), but not in the pituitary. These data indicate that the pattern of up- and down-regulation varies between different tissues and may have relevance for the functional responsiveness to chronic glucocorticoid treatment.

(Supported by NS 41526)

The presence of oxytocin (OXT) immunoreactivity and expression in thymocytes from the thymus of neonatal and adult rats. In adult thymic slices given one of four different steroids (5 mg daily for 3 days) we found several changes in OXT receptors. Testosterone (T) and estradiol (E) both increased OXT receptor affinity, 

\[ K_f = 0.2 \text{nM} \text{ for oil, and 0.12 for E and T, while} \]

receptor densities (Bmax increased 10 fmol/mg protein for oil vehicle, and 5.4 and 6.4 mg E and T). Corticotropin and progesterone (P) reduced receptor densities (Bmax was 75 for both) without affecting receptor affinity. Giving 500 mg/kg 5 hours before decapitation to animals treated with 5 mg E resulted in an increase in the density of thymic OXT receptors increased from day 4 to day 22 of development (from Bmax = 0.266 ± 0.08 to 0.621 ± 0.05 fmol/mg protein). Oxytocin release in adults may be an important factor in establishing immune competence.

497.10 CALCULUM ENHANCES CAMP PRODUCTION IN T LYMPHOCYTES STIMULATED THROUGH THE 8-ADRENERGIC RECEPTOR. S.L. Carlson and T.L. Rossetti*, Dept. of Microbiology and Immunology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0904

Lymphocytes express functional 8-adrenergic receptors that are linked to the cAMP second messenger system. Stimulation of these receptors has been shown to alter lymphocyte activation and function. The goal of this investigation was to determine the effects of 8-adrenergic drugs on T cell function. We have investigated the intracellular signals generated in human T cells in response to stimulation with an 8-adrenergic agonist (isoproterenol, ISO) and a muscarinic (methylycholine, MCH). Dual stimulation of T cells with ISO and MCH results in the synergistic rise in cAMP production (J. Neuroimmunol. 24(155, 1989). ISO alone does not stimulate significant signals but rather signals the cell via the PI cycle resulting in increased intracellular calcium (Ca2+); and activation of protein kinase C.

Increased Ca2+ is one possible mechanism by which PHA stimulation could enhance 8-adrenergic-linked cAMP production. To examine the effect of increased Ca2+, two agents were used that increased [Ca2+]i, by different mechanisms. Isoproterenol acts at the level of the plasma membrane to carry Ca2+ ions across the membrane from the extracellular medium. Thapsigargin, (gift from Dr. Michael Hanley, Univ. Cambridge) acts at the level of the endoplasmic reticulum to inhibit the Ca-ATPase pump, resulting in increased [Ca2+]. Neither agent had an effect on the level of cAMP in T cells when used alone, but when paired with stimulation of the 8-adrenergic receptor caused a synergistic rise in cAMP. These results suggest that both the Ca2+ and the 8-signals result from muben stimulation of T cells are involved in the synergistic activation of T cells in collagen-stimulated T cells. The stimulation of T cells by PHA and ISO in Ca2+-free medium resulted in enhanced cAMP production, presumably because of the release of Ca2+ from intracellular stores. Collectively these results indicate that Ca2+ has a role in modulating cAMP production in T cells. (Supported by NS-17423 and FS-5905891).
497.15


Natural killer (NK) cell function depends on a myriad of molecules that interact with membrane receptors and ion channels. Steroid-like Na+ channel agonists such as veratridine and batrachotoxin depolarize human NK cells in a tetrodotoxin (TTX)-sensitive fashion that suggested the presence of Na+ channels (Mandler, R.R., et al., J. Immunol. 144:1236-1237, 1990). This discovery prompted a study of purified human NK cell excitability to a variety of human steroids using flow cytometry and voltage-sensitive dyes. Highly-purified human NK cells (CD68+>5 x 10^5) were prepared using a negative panning technique. Progestosterone (10-200 uM) depolarized NK cells in a concentration-, time- and temperature-dependent fashion. Neither cortisol, cholesterol, testosterone, 11-B0 cortisone, an progesterone, nor aldosterone modified NK cell excitability. Progestosterone but not the other steroids produced marked effects in NK cell cytotoxicity against K562 tumor target cells. Progesterone effects on purified human NK cell excitability and cytotoxic function might represent one of a number of mechanisms of neuroendocrine modulation of the immune response.

497.17

REJECTION OF MESENCEPHALIC RETINAL THYMOGLOBINS IN THE RAT INDUCED BY SYSTEMIC ADMINISTRATION OF RECOMBINANT GAMMA INTERFERON. I. Subramanian, J.F. Pollack and R.D. Lukas. Dept. of Neurobiology, Anatomy and Cell Sciences and Neurosurgery, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Previous studies have shown that well-integrated mesencephalic retinal thyroglobins can be induced to rejects by various systems, but that the cytokine system induced rejection response is unique in several respects (Pollack et al. Neurosci. Abs. 12:11, 1989). In each of these rejection paradigms the development of major histocompatibility complex (MHC) antigen expression on cells in and around the graft was associated with the ability to reject. To determine whether the induction of MHC antigen expression was itself a sufficient stimulus to provoke a rejection response, we examined the effect of systemic administration of a recombinant gamma interferon (a strong inducer of MHC antigen expression) on xenograft viability.

Post-natal day 5 Sprague-Dawley rats received mesencephalic grafts of embryonic day 13 CD-1 mouse retina. Starting at 21 days of age, one half of those animals received 2 x 10^5 units of recombinant gamma interferon intraperitoneally for 5 to 9 days while the other half served as litter matched controls. All the rats were sacrificed 30 days after the final injection and 30 micron thick coronal sections were made through the mesencephalon. Adjacent sections were stained with cresyl violet and antibodies against rat MHC class I (OX-18) and class II (OX-6) antigens, microglia (OX-42), astrocytes (GFAP) and lymphocytes (anti-lkm).

Each of the animals that received gamma interferon showed a strong rejection response characterized by perivascular cuffing with mononuclear cells in and around the graft, infiltration of the graft and the surrounding host tissue by lymphocytes and MHC class I and class II-antigen positive cells resembling reactive microglia. The rejection rate in this group was significantly higher than the control group (Fisher's exact test, p<0.003). These results suggest that gamma interferon is a lymphokine mediator of the initiation and/or subsequent maintenance of the rejection response to cross-species transplants. (Supported by CMRF 079 and EY 5038 grants)

497.18

DIFFERENTIAL AND SEX SPECIFIC EFFECTS OF KAINIC ACID (KA) AND DOMOIC ACID (DA) LESIONS IN THE LATERAL SEPTAL AREA (LSA) ON BODY WEIGHT (BW) AND THE HUMORAL IMMUNE SYSTEM. L. Wintrebert, J. Burns and D.M. Nance. Dept. of Pathology, Univ. of Manitoba, Winnipeg, MB, Canada, R3E 0W3.

KA lesions in the LSA of female rats increases BW and decreases the humoral immune response, relative to male rats. In rats implanted with KA and potent kainic agonist than KA. We tested the effects of DA lesions by DA (0.15 µg in 0.5 µl) on the immune response of female and male rats immunized with ovalbumin. Similar to KA, DA produced an increase in BW gain and reduced female rats. DA produced greater cell loss in the LSA of male rats than in females. Unlike KA, DA lesions had no effect on the immune response of female rats nor was the immunosuppression of male rats altered by the lesion. We reexamined the effects of KA (0.375 µg in 0.25 µl) in the LSA on BW and the immune response of male and female rats. In both males, KA lesions produced a decrease in BW gain and reduced humoral immunity. Similar lesions in male rats had no effect on BW or immune response. To test if the absence of an effect of DA lesions on the immune system of female rats was due to the small lesion size used in the first study, we tested the effects of more concentrated DA (0.15 µg in 0.25 µl) on the immune response of female rats. This dose produced substantial cell loss in the LSA and a large increase in BW gain, but the immune response of the DA lesioned females was similar to controls. Thus, both KA and DA lesions in the LSA produce an increase in BW gain of female rats, but only KA lesions reduce humoral immunity. BW regulation and immune function were unaltered by DA or KA lesions in the LSA of male rats. Supported by Medical Research Council of Canada.

497.19

BETA ENDOPHIN MODULATES CALCIUM CHANNEL FLUX IN HUMAN NEUROPHILOGS. D.B. MILLAR*, D.B. MADZOR and John Thomas, NIMH Neuroscience Center at St. Elizabeths, Washington, DC 20032 and Naval Medical Research Institute, Bethesda, MD 20814. We report here that Beta-endorphin (Bend) also significantly influences n-formyl-methionyl-leucyl-phenylalanine (fMLP) stimulated Ca2+ entry into human neurophils. Ca2+ flux was analysed using the Fura-2 fluorescent technique and the equations described by Mather and Millar (FASEB J. 3, 44, A1209 (1990)). 10^-5 M fMLP induces a Ca2+ influx in neurophils comprising a sharp initial rise and fall followed by a steady increase. Experiments with Prostaglandin d2, dibutyl cyclic AMP and ETU showed that the second peak was due to influx of Ca2+ into the cell via a Ca2+ channel. Neurophils incubated for 1 1/2 h at 37° with Bend (10^-6 to 10^-8 M) show major changes in the shape and height (f2) of the second peak in a dose and donor dependent manner. In some donors, f2 is reduced by about 50%. Other donors increase values close to that of the initial peak (usually the first peak height is 2x the height of the second peak). Finally, experiments with naloxone, an opioid antagonist, have not yet satisfactorily shown the reactive site for Bend to be an opiate receptor.

Mechanisms underlying the immunologic privilege of the CNS are poorly understood. We have compared serum antibody responses to OVA, administered either into CNS or systemically in Sprague-Dawley rats, to determine if reduced activity of the afferent limb of the humoral immune response was due to a central nervous system (CNS) barrier, or if it was secondary to reduced lymphocyte traffic to the CNS. All serum antibody assays were ELISA, using OVA as antigen. When 90 μg OVA was infused into the cisterna magna, or into the lateral ventricle of Sprague-Dawley rats through a lateral ventricle cannula implanted 7 days previously, no detectable serum anti-OVA antibodies were measured 2 weeks later. This was true regardless of whether the animals received subcutaneous injections of incomplete Freund's adjuvant three days prior to immunization. Thus, the results support a role for the CNS in the immunologic privilege of the CNS. Supported by an N.I.H. grant NS-11050.


We have developed a nonpathologic, nontraumatic rat model in which to study the humoral immune response to virus in the central nervous system (CNS). Fisher rats were microinfused with LCMV through a lateral ventricle cannula implanted 7 days previously. At 21 days, CSF and serum immunoglobulin (Ig) titers and concentrations of IgA and IgG were determined by enzyme-linked immunoassay. Rats infused with cell culture supernatant or saline and sham-operated rats were used as negative controls. As determined by ELISA, IgA titers and index were used to evaluate total CSF IgA and IgG with respect to. CSF-infused rats had an elevated total IgA index and 2 were exhibited an elevated IgA index (x = 25; mean ± 5 SD of controls, n=11). We are investigating whether this specific appearance of IgA within an intact BBB is due to a secretory transport mechanism analogous to that found in mucosal tissues outside the CNS. Supported by NS-11050.

489.3 STUDIES ON THE FUNCTION OF β-ACETYL MURAMYL Dipeptide IN THE INDUCTION OF AUTOIMMUNE DISEASES. F.C. Westall and Ali Mohammadi. Institute for Disease Research, P.O. Box 1293, Alta Loma, CA and California State Polytechnic University, Pomona, CA 91768.

Acyl muramyl dipeptide (MDP) derived from gut bacteria is found throughout the human body. As an adjective, it has been used to induce autoimmun diseases, e.g., experimental allergic encephalomyelitis (EAE) and artificial castration. For induction of disease, the association of MDP with the antigen is quite important. We have examined whether MDP functions by suppressing the proteolytic digestion of the antigen. If potential antigens are digested too rapidly, they would not be available for immunoprocessing. MDP does suppress the digestion of these antigens. However, since both antigens themselves show remarkable stability against the proteases utilized, it would seem unlikely that this is a function of MDP. More probably the MDP-complex is immunoprocessed as a unit. Finally it would appear that digestion of potential immunogens plays an important part in determining what is immunogenic.


Our laboratory has found that extensive damage occurs to descending monoamine- and peptide-containing axons during development of the CNS autoimmune disease experimental allergic encephalomyelitis (EAE). This damage is usually found near perivascular and submeningeal inflammatory foci. The α-adrenergic antagonist, prazosin, suppresses the clinical signs of EAE and delays the inflammatory response in the spinal cord (Bromon et al., Proc. Natl. Acad. Sci. 82:5915, 1985). The present study used immunohistochemical techniques to determine whether prazosin also prevents axonal damage in EAE that have been inoculated for EAE.

Lewis rats were inoculated for EAE with Lewis rat spinal cord homogenate. Prazosin injections began on day 7 postinoculation (2 mg, i.p., twice per day) and continued until sacrifice day (day 15). The control group received equivalent injections of the saline vehicle. The prazosin treatment markedly attenuated clinical signs (hindlimb ataxia was observed in only 1/7 rats, whereas 6/7 control rats developed severe to complete hindlimb paralysis). Inflammation in the spinal cord was attenuated, but not prevented, by prazosin (17.3 ± 1.7 versus 30.2 ± 0.5 foci per 6 mm long section). Prazosin also attenuated, but did not prevent, axonal damage (2.6 ± 0.2 versus 6.8 ± 0.6 SHFT axonal damage foci per 6 mm long section). The close correlation between degree of inflammation and severity of axon damage provides additional evidence that these axons become damaged as they encounter inflammatory foci in the spinal cord.

489.5 ANTIGEN PRESENTATION BY GIALL CELLS FROM EAE RESISTANT AND SUSCEPTIBLE STRAIN OF MICE. G. Birnbaum, L. Albrecht* and G. Eichhorn*. Dept. of Neurology, Univ. of Minnesota Sch. of Med., Minneapolis, MN 55455

We studied the antigen presenting properties of glial cells derived from strains of mice resistant (B10.S) or susceptible (SJL/J) to experimental allergic encephalomyelitis (EAE). Indicators were T cells lines sensitized to myelin basic protein (MBP) that were Class II identical with the glial cells. Mononuclear cells from new born mouse cerebral hemispheres were exposed to a mixture of lymphokines from activated T cells to induce Class II MHC expression on glial surfaces. Glia were irradiated and subconfluent wells of MBP (0.25, 2.5, or 25 μg/ml) were placed onto the cultures along with 1 X 10^5 MBP sensitized T cells. After 48 hours the proliferative responses of the T cells were measured using a [3H]-thymidine incorporation assay. At high antigen concentrations (25 μg/ml) B10.S and SJL/J glia functioned equivalently in inducing MBP sensitized T cells to proliferate. At low antigen concentrations (2.5 μg/ml) MBP, derived from the EAE susceptible strain, SJL/J, were more efficient at presenting antigen as measured by an increased ability to induce T cell proliferation (multiplication factor). Spleen cells from both mouse strains were identical in their abilities to present MBP indicating differences in presentation were tissue specific. One function of glial cells in the CNS may be to present antigens. This study suggests that the CNS myelin autoimmune disease may be the antigen presenting capabilities of the target organ, in this case the brain. Supported by the National Institutes of Health.


Our previous work has shown that CSF-infused albumin induced a significant systemic antibody response to MBP in Sprague-Dawley rats. Present studies showed at evaluating the immune response to a CNS antigen, guinea pig MBP, infused into the CSF of the Lewis rat, a strain resistant to the CNS experimental allergic encephalomyelitis (EAE). The immune response was evaluated by measuring serum antibodies and EAE expression. As a positive control, the immune response to CSF-infused chick ovalbumin (COA) was studied in a separate group. Antibodies (90 or 900μg) in saline (10 or 20μl) was infused (0.5/sal/mm) through a cisterna implanted into a cerebral ventricle. Serum antibodies titers were measured using an ELISA, 16-16 days post CSF-infusion. Titters of anti-COA antibodies were detected in most rats (8/11) and titers ranged from 20 to 2,000. In contrast, serum anti-MBP antibodies were found only in a few rats (3/15) and were low (titer 20). Preliminary studies indicate that rats (N=3) receiving a CSF-infusion of MBP present an antibody response that demonstrably delay an EAE onset (Day 12 v 16) and less severe symptoms (score 1 v 3) compared to naive rats (N=7). Supported by NIGMS, NIMH and NHLBI.
498.7 IMMUNOLOGIC CHANGES ASSOCIATED WITH SPINAL CORD TRANSECTION IN RATS. J.B. Giedraitis*, N.S. Haltt, R.A. Mendez*, M.P. O'Gradyt, M. Wiznogorska* and S. Filipe*. Dept. of Anatomy*, Texas A&M Univ., College Station, TX 77843-1114, and Dept. of Neurology, Div. of Psychiatry, Univ. of South Florida College of Med., Tampa, FL 33613.

The study was designed to determine whether reactive astrocytes in the spinal cord of rats are capable of secreting monokines and lymphokines. Male Sprague Dawley rats ranging in age from 40 to 50 days were anesthetized and a dorsal midline incision was made in the mid-thoracic region. A spinal cord transection was performed at the T5-T6 vertebral level, and the spinal cord was transected with a scalpel. Sham animals were subjected to the same surgery, but omitting the transection of the spinal cord. Transection was performed at the T5-T6 vertebral level, and the spinal cord was transected with a scalpel. Four 2mm sections of spinal cord were dissected from each rat at either 24, 72 hours, 7 days, or 14 days. The spinal cord sections included adjacent and distal sections from both rostral and caudal to the site of transection. Detectable levels of interleukin alpha have not been found in these sections at either 24 or 72 hours, although some positive assays of interleukin activity are currently being assessed along with the later time points. Decreases in thymus and spleen weight were detected at both the 24 and 72 hour time points and these are being correlated with lymphocyte blastogenesis and cytokine production. Since cytokines act upon and are secreted by astrocytes, we are hypothesizing that they produce and play a physiological role within the neurogenic adjacent to the spinal injury. These studies were supported in part by a grant from the NIH, DA05723.


Adjuvant arthritis (AA) was examined in adult male Lewis rats following local sympathectomy (STP) of draining lymph nodes (LN). Rats received bilateral local LN injections of hydroxypropyl-beta-cyclodextrin (6-OH-CD) or vehicle (VEH). Rats were killed 6-9 days after injection of Freund's complete adjuvant (FCA) at the base of the tail. The remaining rats from each group received the same volume of saline, resulting in four treatment groups: 6-OH-CD/FCA, VEH/FCA, 6-OH-CD/VEH. These treatments, AA was assessed in body weight, dorsal and ventral sciatic nerve uptake and scoring and radiographic analysis for day (D) 27. STP of draining LNs accelerated the onset and exacerbated the inflammation and osteopathic changes associated with AA. Body weight gain was significantly less in AA rats compared to the saline-treated group. The difference in AA rats was even more pronounced if saline was instilled into a splinter of the right hind paw. One day after injection of FCA or mineral oil, half of the rats from FCA group and mineral oil group received injections of capsaicin (CAPS) into the right draining LN. The remaining rats from each group were administered the same volume of vehicle, resulting in four treatment groups: FCA/CAPS, FCA/vehicle, AA/CAPS and AA/vehicle. Following these treatments arthritis was assessed by changes in body weight and dorsoplantar swelling for 20 days. Body weights of AA rats, were significantly decreased by 14 days following FCA injection compared to non-AA rats. However, AA rats maintain body weight from day 1 of the experiment through the expression of AA 20 days later. Dorsal inflammation of hindlimbs was apparent by day 20 post-footpad injection in the FCA/vehicle group. No significant difference in arthritis was observed in non-PA treated rats. No significant difference in dorsoplantar widths of any treatment group was observed after controls to the day 20. As AA rats were significantly increased compared to both control groups by day 20. At day 20, a significant increase in left dorsoplantar width was observed only in the non-PA treated group. At day 20, left dorsoplantar widths of the FCA/CAPS group did not differ from either control group. Further studies are required to determine if this difference represents a delayed onset of AA contralateral to the deviated side or an inability of these limits to develop the inflammatory characteristic of arthritis. These findings indicate a dual role of SP interaction in AA through modulation of both inflammation in the joint, and immune responses in lymphoid organs.


Sympathetic noradrenergic (NA) innervation of draining LN play a regulatory role in processing of a relevant antigen for the initiation of AA changes, and that absence of these NA fibers results in exacerbation of the severity and acceleration of the time of onset of AA.

498.12 TUMOR NECROSIS FACTOR ALPHA AND INTERLEUKIN-1 ALPHA INCREASE PAIN THRESHOLDS IN THE RAT. A.E. Panerai, P. Sacerdote, P. Ricciardi, M. Bianchetti. Dept.Pharmacol, School of Medicine, Univ. Milan, Milano, Italy.

Recent evidences show that TNF alpha and IL-1 synthesis is not confined only to cells of the immune system, but is present also in brain. We investigated whether TNF was able to modify pain thresholds in the rat and since TNF induces IL-1 production, we considered this cytokine also. In the hot plate test intracerebroventricular injection of TNF at the dose of 1 and 2.5 ng/rat induces a significant increase in pain thresholds at 3 and 5 min: higher and lower doses are ineffective. 5 and 10 ng of icv IL-1 also increase pain thresholds with a time course similar to TNF. The administration of a m-ab against IL-1 blocks TNF induced analgesia suggesting the involvement of IL-1 in the TNF effect. Anti sera against the endogenous opioid(s) (endorphin, dynorphin met-enk) are ineffective on both cytokines. receptor binding studies it appears that binding is mediated by the opio- id receptor. The cyclooxygenase inhibitor indomethacin does not block the effect of either cytokines. Our data are a further example of the links existing between the nervous and immune system.
PLASMA β-ENDORPHIN (β-E) AND NATURAL KILLER (NK) CELLS IN CONGENITAL INDIFFERENCE TO PAIN (CIP).


CIP is a rare inherited syndrome characterized by unresponsiveness to painful stimuli. We have evaluated β-E and NK cell levels among these patients. Immune function in two patients affected by CIP. Peripheral blood lymphocytes (PBL) were incubated with graded concentrations of phytohemagglutinin (PHA), with or without addition of β-E. Incorporation of "H-thymidine was measured 72 h later. We have also measured plasma levels of β-E, ACTH, and cortisol. PBL proliferation rate was normal, either after incubation with PHA, β-E alone, or in association among the surface antigens studied. NK Leu 7 cells was decreased significantly (p<0.01, t test). Plasma β-E levels of patients were higher (p<0.01, t test) than controls. No change occurred in plasma ACTH and cortisol levels. Results indicate that in CIP high plasma β-E levels are associated to NK cell decrease.

NEURAL-IMMUNE INTERACTIONS: INTERLEUKINS

940.1 NOREPINEPHRINE AND PROSTAGLANDIN E2: EFFECTS ON SINGLE UNIT ACTIVITY RECORDED FROM THE RAT HYPOTHALAMUS IN VIVO. W. Becher, and W. Dafny. Dept. Neurobiology and Anatomy, University Texas Medical School at Houston, TX 77025.

Both prostaglandins and activation of the central nervous system have been associated with IL-1β induction of corticotropin releasing factor (CRF) from the POM into the portal circulation. The goal of this study is to examine the effects of locally applied norepinephrine (NE) and prostaglandin E2 (PGE2) on the electrical activity of single neurons recorded from the anterior hypothalamus/paraventricular area in the anesthetized rat. Male Sprague Dawley rats (280-320 g) were anesthetized with urethane (1.2g/kg iv) and placed in a stereotoxic instrument. The dura was bored, a 3mm hole drilled in the cranium and the dura reflected. Norepinephrine and PGE2 were entraphosed onto individual neurons by means of a multilayer microplette assembly with a recording barrel attached. Preliminary results indicate that the percentage of neurons responding to NE with a change from baseline activity increased with dose (20, 40, 80μg - 50-1.5, 4-6% respectively) and that all doses resulted in excitation. Little change was seen in the overall number of cells responding to a dose of 20μg of POE (20.40, and 50.44 and 54% respectively). The two lower doses resulted primarily in excitation while the highest dose produced inhibition in the majority of responders. Previously we demonstrated that IL-1 itself was capable of exciting these neurons as well. These results suggest that: 1) IL-1β activation of the NE input to the POM may stimulate CRF release and 2) PGE2 induced by IL-1 may also stimulate POM CRF neurons.

940.2 CYTOKINE-ASSOCIATED AXONAL CHANGES COINCIDE TEMPORARILY WITH CIRCULATING TUMOR NECROSIS FACTOR AFTER RIL2 INFUSION. J.M. Bishop and H.G. Chramb, Dept. of Anatomy, Medical College of Virginia, Richmond, VA 23298.

In brains of ril2-infused rats, we have recently reported alterations of axonal ultrastructure reminiscent of those described in murine spinal cord slices treated with tumor necrosis factor (TNF). These changes were accompanied by other neuronal and glial abnormalities similar to those previously reported in various inflammatory demyelinating conditions and nerve injuries by other investigators, to cytokines. In our laboratory, such alterations were seen as early as 4 hours after a single ril2 infusion and were never observed after 3 or 5 days of ril2 infusions, administered 3 times daily. The present study was undertaken to determine whether ril2-treated brain possesses similar CNS functions. Normalized temporally by elevations of circulating TNF. Adult male rats were infused once (N=6) or twice daily for 3 days (N=7) with ril2, N x 10 (IU/kg, Cetus Corp.) Controls (N=15) were compared with saline-infused animals. Brain blood samples were drawn prior to infusion in 16 additional animals and at 2, 6 and 8 hours after the final infusion in 8 saline recipients. The activity of EIA was determined by U929 fibroblast bioassay (kindly performed by Cetus Corp.). In 14 of 16 samples drawn prior to infusion and in all serum samples from animals infused with ril2, TNF activity was below the assay level of detection. serum samples from infused once with ril2 ranged in TNF activity from 34 to 430 ng/ml. Animals receiving ril2 2 or 3 days exhibited TNF activity ranging across animals from 0.31 to 86 ng/ml, except for 1 hr, sample (430 ng/ml). TNF levels in the multiple ril2 infusion group were statistically lower than those measured in the single infusion group. Although TNF activities varied across animals in each group, activities for each animal generally remained constant across sample times. We concluded from these data that, after systemic ril2 administration, elevations of circulating TNF are transiently coincident with the presence, in brain, of axonal changes similar to those previously associated with TNF. The question remains of whether circulating TNF and cytokine-mediated morphological CNS abnormalities are causally related remains to be determined and merits further investigation. (Supported by NS 25871)


This study is a preliminary report of our attempts to determine the nature of immune system involvement in immune system activity is regulated by multicomponent feedback loops between the immune system and the nervous system. IL-1 is suggested to be one substance that conveys information from the immune system to nervous system. Our initial attempt was to measure the IL-1 activity in the following immune system activation using immunohistochemical procedures, but this has not been successful due to lack of antibodies reacting with the rat IL-1 antigen. Since IL-1 has been reported to activate astrocytes, we measured GFAP levels immunohistochemically as a potential indicator of IL-1 activity in CNS. In this study, male Sprague Dawley rats were injected with 10% sheep red blood cells in physiological saline (4ml/kg bwt) to activate the immune system. Control rats were similarly injected but with saline. Vibriomote sections were prepared from animals killed 2 hours, 1 day and 7 days post-injection, and stained immunohistochemically for GFAP. There was no significant change in GFAP staining observed prior to the injection. However, at both 1 and 7 days after the rbc injections there were increases in GFAP staining (about 3-fold). At no time was cortical GFAP staining altered by the injections. This result suggests that astrocytes may participate in neuroimmunological interactions. Supported by the American Heart Asst Grant-in-Aid 890707 and Established Investigator Award 890173 to DGM.
499.5 EFFECT OF INTERLEUKIN 6 ON PROLACTIN SECRETION, cAMP AND INOSITOL PHOSPHATE PRODUCTION IN RAT PITUITARY CELLS. M. Grimaldi*, E. Landolfi*, T. Florio, O. Neucci* and G. Schettini*, Dept. of Pharmacology, II School of Medicine, University of Naples, Via D. Maggiori 5, 80131 Naples, ITALY.

Interleukin 6 (IL6) is a cytokine having many physiological activities. It has been reported that IL6 is released from rat hypothalamus, suggesting a role for this cytokine in the regulation of pituitary hormone release. We analyzed the direct effect of IL6 on cultured pituitary cells by measuring cAMP and inositol phosphate production (PIP2) in response to IL6. IL6 (2000-200 U/ml) inhibited basal, VIP (-24%) and TRH (-20%) stimulated PRL secretion. The addition of IL6 to cultured pituitary cells pre-exposed to IL6 (500 U/ml) for 20 min showed a dose-dependent reduction of the TRH stimulated inositol phosphate production (-25%).

Our data suggest that IL6 modulates PRL secretion and affects the formation of both cAMP and inositol phosphate at pituitary level.


Ulopolysaccharide (UPS) is a bacterial cell wall polysaccharide that mediates fever in the rat. Previous studies in our laboratory have shown that central administration of UPS induces an increase in interleukin-1 (IL-1) levels in the hypothalamus, in addition to stimulating basal hypothalamic TRH bioactivity. In this study, we observed a significant increase in IL-1β mRNA levels in the hypothalamus of UPS-injected rats. UPS-induced IL-1β mRNA was detected within 1 h of injection and peaked at 4 hr. This increase in IL-1β mRNA was accompanied by an increase in IL-1β protein levels in the hypothalamus. These findings suggest that UPS-induced fever is mediated by an increase in IL-1β production in the hypothalamus.

499.9 INTERLEUKIN-1 (IL-1) INHIBITS ACETYLCOLINE BIOSYNTHESIS IN CULTURED BASAL FOREBRAIN NEURONS. L. Ni, R.P. Hart, and G. M. Jonakait*, Dept. Biological Sciences, Rutgers University, Newark, NJ 07102

Since brain levels of IL-1 are elevated in Alzheimer disease (Griffin et al.,PNAS 85, 7611, 1988), we sought to determine the possible influence of IL-1 on cholinergic neurons in both the basal forebrain (BF) and neostriatum (NS). Organ cultures of embryonic rat BF or NS (E16) were grown with and without human recombinant IL-1β (r-IL-1β) for 8-12 days. Cholinergic acetyltransferase (ChAT) activity was measured as an index of cholinergic development. IL-1β (20 U/ml; Cislon) lowered ChAT activity in both BF and NS by an average of 48%. In the BF, IL-1β did not affect levels of mRNA coding for neuron-specific enzymes, suggesting that the effect of IL-1β was not a general toxic action on neurons in the cultures. Moreover, IL-1β lowered mRNA levels on a dose-response curve for glutamate decarboxylase, suggesting that GABAergic neurons were spared the effects of IL-1 and that the neuron that was sensitive was somewhat specific. Cholinergic neurons, though compromised, survived a relatively brief (5-day) exposure to IL-1β since ChAT activity recovered to control levels following a 12-day addition of nerve growth factor following IL-1 removal stimulated ChAT activity still higher. These data suggest that IL-1β specifically affects a significant component of cholinergic development in cultured BF and NS.

499.10 INTERLEUKIN-1 (IL-1) STIMULATES PREPROTACHYKININ GENE EXPRESSION IN CULTURED SYMPATTHIC GANGLIA. G. M. Jonakait and R. P. Hart, Dept. of Biological Sciences, Rutgers University, Newark, NJ 07102

Recent studies from our laboratory have shown that IL-1 dramatically decreases substance P (SP) peptide levels in cultures of sympathetic nerve terminals (SCG; Scholl et al., J. Neurosci. Res. 26:24-30, 1990). Northern blot analysis of RNA obtained from cultured ganglia show that mRNA coding for the prohormone precursor, preprotachykinin (PPT), is increased 4-fold following treatment with IL-1, suggesting that increases in LR3 mRNA levels mediate the rise in peptide. Since ganglia increase SP in response to deafferentation, we investigated the role played by depolarizing stimuli in the IL-1-induced increase in SP. Growth of SCG cultures in 40 mM KCl prevented IL-1-induced increases in either SP peptide or PPT mRNA, suggesting that deafferentation is required for immune responsiveness.

Glucocorticoid hormones suppress immune responsiveness in many systems. In this system, too, dexamethasone inhibited the ability of IL-1 to increase both SP peptide and PPT mRNA (K=5mM).

Nicotinamide adenine dinucleotide (NADH), included in the cultures to block IL-1-induced increases in protaglandins, depressed the IL-1-induced increase in SP only slightly (21%), suggesting that prostaglandins are not primary mediators of this action of IL-1.
499.11 ENDOTOXIN OR INTERLEUKIN-1 ADMINISTRATION ELEVATES PLASMA CORTICOSTERONE, AND BRAIN MHPG AND TRYPTOPHAN
A.J. Dunn, Department of Pharmacology, Louisiana State University, New Orleans, LA 70118-3912.

In previous experiments, administration of endotoxin or infection of mice elicited stress-like elevations of plasma corticosterone, and brain tryptophan, serotonin, and tryptophan hydroxylase activities (NE and 5HT metabolism). Similar results were obtained following IP administration of various preparations of interleukin-1 (IL-1 human or b, or murine a). We have now studied in more detail the biochemical and neurochemical consequences of endotoxin and ICV IL-1 administration. ICV human IL-1b produced dose-dependent elevations of plasma corticosterone, and brain concentrations of tryptophan, serotonin, and the NE hydroxylase (LPH). The effective dose range was not markedly different from that obtained with IP injections. This could imply that the effect of IP IL-1 is not due to penetration of the brain by IL-1. Two different preparations of IL-1, oligolipopolysaccharide (LPS) Sigma L3129 and L3755) administered IP produced dose-dependent elevations of plasma corticosterone. Although responses were somewhat variable, doses >10 mg were always effective, and L3755 was somewhat more potent than L3129. Accompanying the endocrine changes, were elevations of brain MHPG, the serotonin catabolite, 5-HIAA, and tryptophan. There were smaller elevations of the dopamine catabolite, DOPAC. ICV administration of LPS (1.0 to 5.0) also elevated plasma corticosterone, and brain MHPG, but was less effective in elevating brain tryptophan. These results suggest that endotoxin, like foreign antigens and IL-1, can produce stress-like endocrine and neurochemical changes. This most likely occurs by LPS-stimulated macrophage secretion of IL-1.

Supported by grants from NIMH (MH 45270 and MH46261)

499.13 IMMUNOAUTORADIOGRAPHIC LOCALIZATION OF INTERLEUKIN-2 RECEPTORS (Tac Antigen) IN RAT AND HUMAN BRAIN. A. Beaudet, D.M. Araujo, R. Quirion, and P.A. Lapchek, Neuroanatomy Lab., Montreal Neurological Institute and McGill University, Montreal, Quebec, H3A 2B4, Canada.

Recent evidence suggests that the lymphokine interleukin-2 (IL-2) may be involved in the regulation of CNS function. In the present study, we determined the localization of IL-2 receptor immunoreactivity (IR) in sections of rat and human brain using the monoclonal antibody anti-Tac. In rat brain, the Tac IR was localized to a limited number of sites in the hippocampal formation, median eminence-arcuate nucleus complex, cerebral cortex (lamina IV), lateral septum, neostriatum, and cerebellum. This distribution is comparable to that of IL-2-like IR, suggesting that IL-2 may be synthesized and/or stored in the vicinity of interaction with its receptor. The distribution of Tac IR may be due to localization of Tac, or the Tac IR may be detected throughout the hippocampus and dentate gyrus. Moreover, brains from patients with Alzheimer’s disease exhibited qualitatively higher immunoreactivity of the dentate gyrus than those of controls. In summary, our results demonstrate that IL-2 receptors are present in both normal and diseased neocortical brain. These results suggest that IL-2 may be involved in immune responses during neurodegenerative processes, in addition to playing a role in the regulation of neuronal function in the CNS.

Supported by NRC, Canada and FRQ, Quebec.

499.14 IMMUNOHISTOCHEMICAL DETECTION OF INTERLEUKIN-2 IN NORMAL MOUSE BRAIN. P. Villeneuve, J.M. Girard, T. Owen, and A. Beaudet, Neuroanatomy Lab., Montreal Neurological Institute, Montreal, Quebec, H3A 2B4, Canada.

Previous studies have suggested that interleukin-2 (IL-2), a lymphokine produced by activated T cells and playing a central role in immune cooperation mechanisms, may be present in mammalian brain. In the present study, endogenous IL-2 was detected by immunohistochemistry in the brain of normal CD-1 mice. Labeling was performed on frozen sections using a rat IgG2a anti-IL-2 (5J4) as primary antibody and a peroxidase anti-peroxidase or immunoperoxidase as detection procedures. In brains fixed by intracardiac perfusion of a 4% paraformaldehyde solution, dense IL-2 immunoreactivity was detected within the arcuate nucleus-medial eminence complex of the hypothalamus as well as over capillary walls throughout the brain. Animals perfused with a mixture of 4% paraformaldehyde and 0.2% or 0.5% glutaraldehyde exhibited even stronger labeling of the arcuate nucleus-medial eminence complex of the hypothalamus, as well as within capillary walls throughout the brain. These results suggest that IL-2 immunoreactivity in adult mouse brain and indicate that most of the lymphokine is concentrated within the arcuate nucleus-medial eminence complex of the hypothalamus. Supported by NRC.


The cytokine interleukin-1 (IL-1) has been reported to have a number of effects in brain including induction of fever, alteration of slow-wave sleep and neurotransmitter effects such as activation of the hypothalamic-pituitary-adrenal axis and inhibition of the hypothalamic-pituitary-gonadal axis. In this study, we sought to identify the brain areas that might mediate some of these effects of IL-1 by using in situ hybridization of 35S-labeled and from a full-length murine T cell IL-1 receptor cDNA to investigate the distribution of IL-1 receptor (IL-1-R) mRNA in the mouse brain. In general, the level of IL-1-R mRNA signal was very weak in the molecular layer of the dentate gyrus of the hippocampus providing only the prominent signal in brain. Notably, the signal in the hippocampus was much higher than that found in the hypothalamus. IL-1-R mRNA probes displayed no signal above background. The pattern of distribution of IL-1-R mRNA in brain was similar to the autoradiographic distribution of 125I-labeled IL-1 receptor. These results suggest that IL-1 may alter neurotransmitter activity to a large extent through actions in the hippocampus a neural system similar to glucocorticoid regulation of hypothalamic hormone secretion.


The cytokine interleukin-1 (IL-1) has a variety of effects in brain including induction of fever, alteration of slow-wave sleep and alteration of neurotransmitter activity. The potential sites of action of IL-1 in brain were examined using 125I-labeled recombinant human IL-1a (125I-labeled hIL-1a) to characterize IL-1 receptors in homogenates of mouse (C57BL/6) hippocampus and to localize IL-1 binding sites using autoradiography. The binding site, IL-1a, was linear over a wide range of membrane protein concentrations, saturable, reversible, and of high affinity (Kd: 14±4 nM; Bmax: 2.6±1.4 fmol/mg protein). In competition studies, recombinant human IL-1a, recombinant human IL-1b, and a weak IL-1b analog inhibited 125I-labeled IL-1a binding to mouse hippocampus in parallel with their respective binding activities in the brain. The autoradiographic localization studies revealed very low densities of 125I-labeled IL-1a binding, with highest densities present in the molecular and granular layers of the dentate gyrus of the hippocampus and in the choroid plexus. The identification of IL-1 receptors in brain with characterization of brain neurotransmitters and neuroendocrine tissues provides further support for a physiological role for IL-1 to regulate central nervous system activity.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

THURSDAY PM NEURAL-IMMUNE INTERACTIONS: INTERLEUKINS 1213
050.1 LAMINAR DISTRIBUTION OF ZINC IN THE RAT SI BARREL CORTEX. N. D. Ahkbar and P. V. Land, Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261. We used the selenium method of Danscher (Histochemistry 1982 76:281-293) to localize zinc storage granules in the somatosensory (S1) barrel cortex. The distribution of selenide-precipitated zinc was compared with staining for cytochrome oxidase activity. There was a heterogeneous distribution of zinc staining in rat S1 cortex. The darkest staining is in lamina V which stains non-uniformly for zinc. Lamina V contains darkly stained or un-stained zinc staining that tends to fall below the septae between barrels in lamina IV. Lamina IV itself is organized into lightly stained patches, each of which is centered upon a barrel. Intervening septal regions are more darkly stained. A band of light staining is present at the lamina V/IV border. Lower lamina VI and lamina II/III have moderate levels of staining, whereas lamina I is only lightly stained. Several studies suggest that zinc is released at excitatory cortical synapses. In addition, it is thought that zinc modulates neurotransmission at glutamate receptors. Thus the pattern of zinc staining in the barrel cortex may reflect functional differences in excitatory input to different regions within S1. (Supported by NIH grant NS05773 and NIH grant RS 23047).

050.2 METABOLIC CORRELATES OF SENSORY DISUSE AND RECOVERY DURING AGING. J. Metzler and P. J. Land, Dept. of Animal Biol., Univ. of Penn. Sch. Vet. Med., Philadelphia, PA 19104. Effects of chronically altered sensory input on the aging CNS and the time course of any changes examined. The rat vibrissal SI barrel model was used. Effects of disuse and subsequent recovery of single vibrissae (S) on gait and locomotion were examined. S1 functional representations in lam. IV of SI was examined using the 2DG-deoxyglucose (2DG) method. Twenty-four young adult (28-30 day old) and 6 aged (1.5-2 yr old) rats were studied. Two groups of 12 YA had C3 clipped (disuse) unilaterally for 30-90 da. Control side C3 was clipped or not during this time. In 6 animals in each TA group, C3 regrew (recovery) for 30 da. (Surrounding) B1, B2, C4 and D4 vibrissae were spared and stimulated or not following 2DG injection. Of 6 AC rats examined, C3 was clipped unilaterally -4 for 30 and 2 for 90 da; 3 rats had B3, C2, C4, and D3 stimulated during the 2DG procedure. Analyses indicated that in TA and AC animals 1) B3, C2, C4, D3 vibrissae activation produces increased labeling in the "dissused" C3 representation as compared to control side; 2) 30 and 90 da disuse effects are similar; 3) labeling was similar in control and "recovered" C3 representations; and 4) compensatory changes are greater in AC rats. In conclusion, while the TA CNS is capable of greater functional responses to sensory disuse and recovery, the AC CNS also exhibits plasticity. (Supported by U. PA. Res. Found. Grant & USPHS Grant NS-2283.)

050.3 VIBRISSA DEGENERATION EFFECTS ON CORTICAL REPRESENTATION OF SUPRAFOCAL (SOG) AND INFRAFOCAL (IOG) GUARD HAIRS IN RAT P.J. Hend, S. S. Huang, and P. J. Land - Dept. of Animal Biol., Sch. of Vet. Med., Univ. of Penna., and Dept. of Anat., Natl. Yang-Ming Med. Col., R.O.C. Does peripheral degeneration of the principal cortical target (PCT) of a spared sensory receptor organ, which expanded its representation into denervated territories, affect the distance from a distance from a surgical fiber. Six 2-day old rats received unilateral vibrissa denervation by post-denervation, 14C-deoxyglucose (2DG) was injected and either SOG (n=5) or IOG (n=3) stimulated bilaterally. SOG and IOG activation on the non-denervated side were observed as a cortical hypermetabolism. 2DG labeling [cerebral glucose utilization rates (dpm/cm) and areal extent] was observed within as follows: 1) P4/P5; 2) Lam.IV and lam. V/VI posterior-medial barrel subfield (FMRF) and adjacent SOG/IOG PCTs. Results (in comparison to control side) were as follows: (1) (SOG and IOG activation post-denervation 39.5% increase in 2DG in caudal-half of FMRF overlying rows N-E and associated "straddling" labeling beyond their PCT was 45% (SOG) and 119% (IOG); (2) SOG and IOG labeling in registry with the PCTs exhibited no LCGU differences (p>0.5); and (3) mean areal extent of labeling associated with PCTs were altered: 40% decrease (SOG) and 44% increase (IOG). Thus a peripheral receptor organ's PCT representation can be affected (e.g., areal extent altered) by a "Distant peripheral degeneration and may significantly impact the cortical processing. (Supported by USPHS grant RS-22823 and R.O.C. Natl. Sci. Council Grant)

050.4 DEVELOPMENT OF LAYER IV IN RAT SOMATOSENSORY CORTEX L. Zhang and N. G. F. Cooper, Dept. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38163 Layer IV of the somatosensory cortex of rodents contains a characteristic pattern of neuronal aggregates called barrel subfields. The objective of this study is to relate patterns of thalamocortical afferents to barrel subfield patterns during development. We have investigated the development of the rat somatosensory barrel field cortex with cresyl violet and carboxyamine dye Dil as follows: 1) Cresyl violet staining of tangential sections of rat pups; 2) postnatal day 2(P2) through P11. Thalamocortical mapping with Dil in postmortem fixed tissue from P1 (day of birth) to P9. We found that granule cell labeling can be detected in a continuous sheet prior to P9. On P4 separations in this sheet allowed visualization of cortical subfield representing face, forelimb, hindlimb and trunk. Nisol stained barrels were detected on P4 in the face area. Barrels representing forelimb and hindlimb were visible between P5-P6. Dil labeled thalamocortical axons reached the marginal zone on P1 and appeared to be sorted in layer IV of the cortical-face region by P3. Thus, the pattern of thalamocortical afferents from VPM appears before the corresponding cortical barrels representing the face. Further studies are required to determine if this sequence is repeated for forelimbs and hindlimbs. The segregation of thalamocortical axons appears to be concurrent with the appearance of glial patterns, but not with neuronal patterns. Supported by NIH-ME, EY0709.

050.5 THALAMIC AFFERENT SEGREGATION PRECEDES BARREL FORMATION IN THE RAT SI CORTICAL CORTEX. R. J. Rezvani and J. Havell, Dept. Brain & Cognitive Sci., M.I.T., Cambridge, MA 02139. The formation of periphery-related patterns by somatosensory projection to the thalamus differens was examined in paraformaldehyde-fixed embryonic and neonatal rat cortex. Axons from the ventrobasal (VB) nucleus to somatosensory S1 cortex were labeled with the fluorescent tracer Dil. VB axons were present in the present cortex (CP) by E19. On PND 0 (E22), layers V and VI have differentiated. By PND 29, VB axons form a dense plexus within these layers; a few then traverse the CP and extend into the marginal zone. By PND1, VB axons define their projection zone in the lower part of the CP, then extend from the VB axons. VB axons form a dense plexus within these layers; a few then traverse the CP and extend into the marginal zone. The VB axons are labeled with the fluorescent tracer Dil and their projections are examined with adjacent flattened cortex preparations. Normally, the vibrissa-related pattern of VB axons emerges on P2. In P2-tated rats, the development of this pattern lags one day behind controls. The density of 5-HT axons in the VB is low in both groups, indicating that the effect is mediated at a cortical level. These results along with previous findings of a delay in the formation of barrel-related patterns suggest a trophic influence by serotonin on maturing VB afferents and on the layer IV cells that comprise the barrels. Supported by NIH grants NS27678 and NS28208.
500.7

Physiological aspects of the barrel cortex of the mouse: a single unit laminar latency analysis.

E. Wilke, M. Armstrong-James and H.-V. Van der Loos.

* Institute of Anatomy, University of Lausanne, Rue du Buginon 9, 1015 Lausanne, Switzerland.
* Dept. Physiology, London Hospital Medical College, London E1 2AD, U.K.

Our previous studies have shown that surround receptive fields of barrel cells in rat SI cortex appear to be constructed intracortically. We now report that the centre receptive vibrisa (CRV) data is relayed vertically in a column of neurons prior to relay to adjacent columns. Extracellular recordings were made in identified layers of rat barrel cortex and latency differences of neurons to stimulation of centre and surround receptive vibissae (CRV and SRV) measured. Latency differences for pairs of centres and surround stimulations of the CRV were also collected. To the CRV, Layers IV and Vb neurons discharged earliest, and layers II and III on average 2.3 ms later. The data suggests that serial relay from layers IV to II is the most common event. No significant differences were found for latency or magnitude of response of layer Va cells to stimulus of centre and immediate SRVs. Temporal summation of responses to several simultaneously stimulated vibissae is therefore ideal in layer Va. Layer II, III, IV and Va cells showed no statistical difference in latency of discharge to SRV, suggesting parallel-column relay for construction of surround receptive fields. The mean transmission velocities were calculated at about 0.08 m/s for column-column information transfer and 0.18 m/s for column-layer (layers IV-II) transfer (assuming major relay by layer IV).
500.13

Retrograde tracing experiments were conducted to localize Nucleus Basalis Magnocellularis (NBM) neurons projecting to somatosensory cortex barrels. Barrels were mapped under physiologic control using a tungsten microelectrode. Rhodamine dextran was injected into one barrel per rat. Rats were sacrificed 3 to 10 days later. Cytochrome oxidase staining confirmed barrel field injection sites encompassing no more than 2 barrels. Thalamic ventrobasal complex (VB) cells labeled intensely to all rats. Labeling of NBM neurons was less intense and improved with longer post-injection survival times. Labeled neurons consistently occupied a region of anterior NBM extending caudally approximately 1.5 mm from the anterior commissure. Labeled cells within each coronal section were located within the ventral portion of NBM, 1.5 to 3.0 mm from the midline. The maximum number of labeled cells per coronal section was 7 and 28 for NBM and thalamic neurons respectively. Total number of labeled NBM neurons was variable (range 1-4) despite consistent labeling of BV neurons.

Barrelfield projecting neurons of NBM are located within a discrete region of the nucleus and may be selectively manipulated with a stereotaxic probe. (Supported by NIH Grant NS08438-01).

501.1

Genetic ablation of gamma-2 crystalline expressing cells leads to reductions in lens size and these animals animals have a microphthalmic phenotype (Breitman et al. Science 238: 1987). In order to evaluate the effects of microphthalmia on the number and distribution pattern of retinal ganglion cell layer (GCL) neurons, cell counts and isodensity maps were made from flat-mounted retinas of control (CD-1) and transgenic microphthalmic mice. Isodensity maps revealed a centroperipheral gradient with the highest cell density just temporal to the optic disk and the lowest in the far periphery. The cell density was lower in microphthalmic mice than controls at all retinal eccentricities. Estimates of the total number of neurons in the GCL revealed that there were 70% fewer neurons in the microphthalmic GCL compared to control (231,000 vs 157,000). The distribution pattern revealed that transgenic microphthalmic mice possessed more optic axons than controls (8,500 vs 52,500). Comparison of these studies suggests that a high proportion of the neurons in the GCL of transgenic animals are displaced amacrine cells.

501.3

Nerve ganglion cells in the neotenic tiger salamander retina were examined morphologically by injection of horseradish peroxidase (HRP) into the optic nerve sheath, followed by computer aided neuron reconstruction and cell counts. Based on cell size, position and dendritic complexity, two broad types were identified and correlated to date (small-simple, SS; small-complex SC; large-simple LS; large-complex LC). Cells having an area of influence of less than 30,000 μm² were considered small. Cells with less than 35 terminal dendritic branches and/or short dendritic segment length (less than 15 μm) were considered simple. LC cells had a circular dendritic branching pattern extending within the inner plexiform layer (IPL). The dendrites of LS cells branched in a polar pattern and were considered to be sub-lamina a and/or b of the IPL. SS and SC cells arborized in both laminae. SS cells differed from SC cells in their overall dendritic fiber length and a smaller average number of terminal branches (less than 4; 5 respectively). The density of ganglion cells was estimated at 350 cells/mm². Soma density and size were independent of eccentricity. Displaced ganglion cells were rare (2% of SS). These findings show that the ganglion cells of these specimens differ dramatically from those of the misateur retina. Detailed structure/function correlations will be necessary to further characterize ganglion cells in these animals. (R01EY07376)

501.4

Displaced ganglion cells (DGCs) represent the sole source of retinal axons entering the nucleus of the basal optic root (nORB) of the avian accessory optic system. In this study we investigated chemically-specific DGCs projecting to nORB. Retrograde labeling of DGCs was obtained with rhodamine beads injected into nORB of pigeon anesthetized with ketamine and xylazine. The indirect immunoperoxidase and avidin-biotin methods were used to detect immunoreactivity to antibodies against substance P (SP, Sara Lab) and cholecystokinin (CCK, Immunonuclear). Following chemically-specific injections into nORB, approximately 4,700 DGCs were retrogradely labeled in the contralateral retina. About 25% of these DGCs were also labeled with the antibody against SP. A different population of about 15% of the DGCs projecting to nORB showed CCK immunoreactivity. Both types of DGCs were mainly found in peripheral retinal regions and their sizes ranged from 12-24 μm (SP) and 18-29 μm (CCK). Together with previous data on catecholaminergic DGCs, the present results indicate that retinal inputs to nORB are chemically heterogeneous.

Supported by FAPESP, CNPq and FINESP (Brazil).
Sensory Society

the Fourier off that irregularities tested bipolar irregularities, the majority pig anesthetized AB5, have THURSDAY

the UV-on/red-off labels, which reported, show the single-unit response to both applications of agonists and antagonists in both current- and voltage-clamp. All cells displayed an inward current to NMDA when in normal (1 Mm) external Mg\(^{2+}\) and held at -60 to -70 mV. This current reversed near 0 mV and had a negative slope conductance region beginning at potentials around -30 to -40 mV. Reducing external Mg\(^{2+}\) linearizes the current-voltage relation and increases both baseline current noise and NMDA-evoked currents while increasing external Mg\(^{2+}\) reduces noise and blocks NMDA currents.

Application of D-2-amino-4-phosphonobutyric acid (D-AP5), 7-chlorokynurenate, zinc or MK-801 had two effects: 1) Responses to NMDA were blocked and 2) Baseline current noise (especially in Mg\(^{2+}\)-free) was reduced in amplitude. Responses to kainite were not affected by any of these agents.

Our results suggest that the external concentration of Mg\(^{2+}\) may play a role in controlling the extent to which NMDA receptors contribute to light-evoked responses in ganglion cells, especially near threshold.

Our data suggest the NMDA receptor of the tiger salamander retinal ganglion cell possesses: a voltage-dependent Mg\(^{2+}\) block of the channel, a strychnine-insensitive glycine site, a zinc site and a phenecyclidine/MK-801 site making these amphibian NMDA receptors similar to those in mammalian CNS neurons. (EY01014)

**501.7**

Neural Coding of E-Vector and Color in Ganglion Cells of Salmonid Fishes...Craig W. Havryshyn...Department of Biology, University of Victoria, P.O. Box 1700, Victoria, B.C., CANADA V8W 2Y2.

Studies have shown that fish can discriminate E-vector and orient to polarized light fields. However, these studies were conducted when little was known about ultraviolet (UV) sensitivity. Our recent studies have shown that UV-sensitive cones in addition to green- and red-sensitive cones play a major role in polarization sensitivity. The UV cones show a preference for vertical E-vector while the green and red cones both prefer the horizontal E-vector. This condition is necessary for discriminations on the basis of E-vector. Since the optic media of fish transmits UV radiation, a linearly polarized UV stimulus can stimulate all the cone mechanisms that overlie the UV spectrum. Single-unit recordings have shown that UV/red opponent ganglion cells exhibit orthogonal polarization sensitivity. In one case, a UV-on/red-off unit was examined. When the UV polarized light stimulus was used under conditions of parallel sensitivity in the UV and red cones, a vertical E-vector orientation resulted in an off response, a horizontal orientation resulted in an on response. Supported by NSERC(UFO403984).

**501.9**

The Role of Ganglion Cell Dendritic Architecture in Increasing the Spatial Bandwidth of Cat Retinal W-Cells...M.H. Row's and J.E. Con...Neurobiology Program, Ohio Univ., Athens, OH 45701.

The contrast sensitivity profiles of many cat retinal W-cells include a significant high frequency shoulder which makes it difficult for them to be fitted with a single gaussian function, and which indicates that the receptive field center mechanism itself is not gaussian in shape. We have used a model of the spatial sampling characteristics of ganglion cell dendrites to show that incomplete sampling of the underlying bipolar array by the ganglion cell could provide one possible explanation for this phenomenon. Given reasonable values for the size and spacing of bipolar cell receptive fields, it can be shown that incomplete sampling of the bipolar array within the domain of a ganglion cell dendritic tree will result in a sensitivity profile for the receptive field center which contains significant irregularities, i.e. a bumpy gaussian. The Fourier transform of such a sensitivity profile produces a contrast sensitivity function which contains a high frequency shoulder very similar to those observed in the spatial sampling of W-cells, and this is true even when the irregularities are confined to the periphery of the dendritic tree. When applied to a camera lucida drawings of HRP filled W-cells, the model produces 2 dimensional receptive field profiles which also contain significant irregularities, and whose fourier transforms include high frequency shoulders at some orientation. Thus, one consequence of incomplete sampling of the bipolar array is to extend the spatial bandwidth of the ganglion cell to frequencies about 30% higher than would be predicted from the overall dimensions of the receptive field. Supported by grants EY00013 and EY08038 from the National Eye Institute.

**501.6**

NMDA-evoked responses in retinal ganglion cells of the larval tiger salamander, L. Sprague & P.A. Coleman...Dept. of Physiology & Graduate Program in Neuroscience, Univ. of Minnesota, Mpls, MN 55455.

We measured N-methyl-D-aspartate (NMDA) evoked currents via whole cell recordings in a retinal slice preparation using the larval tiger salamander. We recorded ganglion cell responses to both applications of agonists and antagonists in both current- and voltage-clamp. All cells displayed an inward current to NMDA when in normal (1 Mm) external Mg\(^{2+}\) and held at -60 to -70 mV. This current reversed near 0 mV and had a negative slope conductance region beginning at potentials around -30 to -40 mV. Reducing external Mg\(^{2+}\) linearizes the current-voltage relation and increases both baseline current noise and NMDA-evoked currents while increasing external Mg\(^{2+}\) reduces noise and blocks NMDA currents.

Application of D-2-amino-4-phosphonobutyric acid (D-AP5), 7-chlorokynurenate, zinc or MK-801 had two effects: 1) Responses to NMDA were blocked and 2) Baseline current noise (especially in Mg\(^{2+}\)-free) was reduced in amplitude. Responses to kainite were not affected by any of these agents.

Our results suggest that the external concentration of Mg\(^{2+}\) may play a role in controlling the extent to which NMDA receptors contribute to light-evoked responses in ganglion cells, especially near threshold.

Our data suggest the NMDA receptor of the tiger salamander retinal ganglion cell possesses: a voltage-dependent Mg\(^{2+}\) block of the channel, a strychnine-insensitive glycine site, a zinc site and a phenecyclidine/MK-801 site making these amphibian NMDA receptors similar to those in mammalian CNS neurons. (EY01014)

**501.10**

Oscillations in Cat Retinal Ganglion Cell Responses to Light Flashes...A.W. Przybylski...Dept. of Physiology, Freie Universit\"at Berlin, Arnimallee 22, 1 Berlin 33, DDR.

The action potentials of ganglion cell (GC) responses to diffuse light flashes were convoluted with the second derivative of the Gauss function (Mexican hat) to obtain wavelet transformation. The width of the Mexican hat function was varied in 640 (OFF-center GCs) or 512 (ON-center GCs) steps from 0.6 to 384 or 307 ms, which gives different narrow band pass filters. This kind of analysis demonstrates a "mathematical" microscope, whereby the degree of magnification was varied. Some parts of the wavelet transformation were extended and analysed in the frequency domain to explore oscillatory components of the impulse pattern. The amount of different oscillations was richer in class I (Y) than class II (X) and in OFF- than ON-center GCs. The range of analysed oscillations was between about 1000 and several Hz. Increase of the diffuse flash light intensity in the photopic range (0.5 to 100 cd m\(^{-2}\) caused a shift in the frequency of some oscillations, e.g. in the primary activity of ON-center GCs and in the secondary peaks when the irregularities are confined to the periphery of the dendritic tree. When applied to a camera lucida drawings of HRP filled W-cells, the model produces 2 dimensional receptive field profiles which also contain significant irregularities, and whose fourier transforms include high frequency shoulders at some orientation. Thus, one consequence of incomplete sampling of the bipolar array is to extend the spatial bandwidth of the ganglion cell to frequencies about 30% higher than would be predicted from the overall dimensions of the receptive field. Supported by grants EY00013 and EY08038 from the National Eye Institute.
501.11 AN ANALYSIS OF CAT GANGLION CELLS OSCILLATIONS BEFORE AND AFTER INTRAVITREOUS INJECTION OF AMINO- PHOSPHONIBUTYRIC ACID (APB).
T. H. Chung, O. J. Griffith and A. W. Prokofiewski. Dept. of Physiology, Freie Universit"at Berlin, Arminmale 22, 1 Berlin 33, BDR (supported by ENA). We compared the effects of APB injection (0.5-3mg pro eye) into the vitreous body on ganglion cell activity evoked by diffuse short (10ms) or longer (1s) light stimuli or eyeball deformation. The experiments were performed on anaesthetized cats. Single ganglion cell activity was recorded by means of microelectrodes from optic tract axons. In an on-center GC light-induced responses were completely inhibited one to two hours after APB injection. GC response to eyeball deformation was at first reduced and later completely disappeared significantly faster then the responses to light. This could be explained by the fact that APB also acts on synapses between horizontal and bipolar cells (Soc. Neurosci. Abstr. 15, part 1. p. 925,1989). We used wavelet transformation (see our abstract this meeting) to find oscillations in the response of GC to diffuse light flashes before and after APB injection. In some experiments the primary phase (maximum frequency of discharges) was strongly reduced in frequency after APB injection (from 1000-800Hz to 200-200Hz in 20-30min after APB injection). This reduction was not always synchronized with a disappearance of the GC's response to light. This supports the hypothesis that APB also acts on other synapses than photoreceptors - on bipolar cells as in lower vertebrates (Slaughter & Miller 81,86, Shells & al.81, Navy & Copenhagen, 87).

To broaden understanding of the comparative functions of visual-cortical neurones and move toward an ecologically inspired understanding of the neurophysiology of vision, maps were made of the receptive fields (RFs) of single units (n=55) in area VI of five grey squirrels (Sciurus carolinensis). RF maps were made with an on-line computer system that presented a flashed or moving disc stimulus to 16 selected recording sites in a manner previously used to map units in cats, monkeys and hamsters. The RFs could be classified by gross response properties as simple (65%), complex (22%), or unclassified (13%). They could also be classified by their spatial structure as Disc (40%), Bar (11%), Composite (29%). Diffuse (20%) for unclassified (8%). RFs classified Diffuse were always complex, Bar and Disc were always simple, while Composites were either complex or simple. The details of the spatial structure of the complex class vary across species. It is suggested that this class of RFs reflects species adaptation to environmental niche. The maps will be compared to those made in other species.

502.2 SPATIAL AND TEMPORAL PROPERTIES OF CELLS IN THE RABBIT'S STRIATE CORTEX. Cazzonova C, M. Mochtoskoff S, McKenney P.A., Motrin C.*, Nault B.*, Michael Y, School of Physical and Occupational Therapy, McGill University, and University of Montreal, Montreal, Canada.
Bars and spots have been primarily used to determine the visual properties of cells in the rabbit's visual cortex. Our knowledge, of drifting sinusoidal gratings have never been utilized to study the properties of single cortical cells despite the fact that gratings have been commonly presented to rabbits in discrimination tests (e.g. Van Hof et al., 1983). Tungsten-in-glass microelectrodes were used to record visual responses of cells in the striate cortex to drifting sinusoidal gratings of different contrast in rabbits. A total of 35 cells were recorded in the visual cortex. Out of these, 26 units responded to drifting gratings, mostly with modulated discharges. More than half of the units were tuned to low spatial frequencies (SF) (mean= 0.16 c/deg; bandwidth = 2.2 octaves). The remaining cells shown no attenuation of their responses to very low SF (low-pass cells). Out of these low-pass cells, two were more responsive to a full-screen flicker rather than to the drift of a sine-wave grating. Almost half of the cells were tuned for a wide range of temporal frequencies (mean=3.5 - 6.6 Hz; bandwidth around 2.5 octaves), and the remaining cells were low or high-pass (6 and 2 units, respectively). Attempts have been made to record from all part of the visual field. So far, no differences have been noted between the cells' properties with respect to receptive field location (optic axis or binocular area of the visual field). Supported by MRC, FCAR.

We previously characterized the response properties of lagged and non-lagged X and Y cells in the cat lateral geniculate nucleus (J. Neurophyysiol., July 1990). Here, we are investigating their possible influence on visual cortex. Cortical single cells were tuned with narrow bars whose luminance was modulated sinusoidally at several temporal frequencies. First harmonic amplitude and phase were measured at a series of positions across the receptive field. By comparing the spatial filters in each direction at various spatial and temporal frequencies to characterize cells' directional tuning. Lagged and non-lagged geniculate cells differ in phase response: lagged cells have a quarter-cycle phase lag and lags "tens" longer in average. Cortical receptive field subregions similarly fall into groups separated by a quarter cycle phase lag and a 70ms latency increase. The field responses of lagged cells were not as clear as that between lagged and non-lagged geniculate cells, but most simple cell subregions can be classified as lagged or non-lagged type according to their response phase. Some cells in posterior regions were non-lagged, but other cells show both types of subregions. The general data suggested that lagged and non-lagged inputs to cortex might converge to produce direction-specific cells. From simulations, such cells would be expected to show direction selectivity only at low temporal frequencies, below 40Hz, while the output would follow their quasi-cycle phase difference below 40Hz and the non-lagged input would dominate at high temporal frequencies. Area 17 neurons often display these predictions. The loss of direction selectivity at higher temporal frequencies could reflect the loss of high-contrast response phase across the receptor field, as can the cell's spatial frequency tuning. We conclude that lagged input from the LGN may be physiologically demonstrated in visual cortex and may contribute to direction selectivity, and spatial and temporal frequency tuning. Supported by MH47273, EY06634 and EY06459.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990 THURSDAY
502.5 EFFECTS OF NORADRENALINE ON VELOCITY TUNING AND DIRECTION SELECTIVITY OF CAT VISUAL CORtical NEURONS. L. Miesyan, C.S. Lin, B.D. Waterhouse, Dept. Physiol. and Biophih., Hahnemann Univ., Phila., PA 19102

The effect of noradrenaline (NE) on the responses of striate cortical neurons was studied using microiontophoresis techniques in anesthetized, paralyzed cat. Unit responses to optimally oriented bright and dark bars moving back and forth across the receptive field were measured at seven different speeds. Velocity tuning curves were then computed by taking the average maximum response for each speed, direction and bar contrast. A mean index was also calculated for responses to bright and dark bars. In the presence of NE (50 nA) the cells examined demonstrated a shift in their velocity tuning curve such that the cell's preferred velocity was changed, while the shape of the velocity tuning curve was unaltered. In some cases the shape of the tuning curve was also modified during NE application such that the type of velocity response was changed, e.g., velocity broad band converted to "velocity low pass." In the majority of cells studied, the mean direction index increased in the presence of NE. For other cells, the velocity tuning and direction selectivity were unaffected by NE administration. Additional experiments on the same population of neurons revealed no obvious changes in orientation tuning. These results demonstrate that the spatiotemporal properties of striate cortical neurons are, at least, partially subject to modification by NE. In a broader context, these findings add a new dimension of specificity to the potential modulatory influence of the noradrenergic system on central processing of sensory signals. (Supported by AFOSR 87-0318 and an award from the Klingenstein Foundation to BDW)


Stimulus specific response properties were studied intracellularly in cells recorded from cat primary visual cortex. Animals were anesthetized with a gas mixture of 70% N2O and 30% O2, cannulated and paralyzed. In all five direction sensitive complex cells recorded, visually evoked EPSPs were tuned to one direction of stimulus movement. Cross-correlograms of these EPSPs, and EPSPs to the opposite direction were smaller than those to the preferred direction. Visually evoked IPSPs were also tuned to the preferred direction. These results suggest directionally tuned EPSPs may contribute to direction selectivity in complex cells. In an orientation selective simple cell, both EPSPs and IPSPs were tuned to an optimally oriented stimulus. A stimulus oriented diagonally to the optimal orientation evoked EPSPs but they were substantially smaller than those to an optimally oriented stimulus. We suggest the possibility that in the cat primary visual cortex excitatory inputs are tuned to optimal stimuli to produce direction/orientation specific responses and IPSP evoked by non-optimal stimuli contribute to sharpening the direction/orientation tuning.
503.12 COLOR FACILITATES MOTION CORRESPONDENCE IN VISUAL AREA MT
K.B. Dobkins and T.D. Albright. The Salk Institute, La Jolla, CA 92037
A wealth of anatomical and physiological data suggests that motion and color are processed in separate channels in the primate visual system. While psychophysical data on this subject have been more equivocal, recent experiments have shown that color discriminates motion correspondence in an apparent motion display (Green, Porac, and Psycholophy, 45, 159. 1989). Papathomas et al. (1986, 1989). We have used a similar paradigm to investigate the neural basis of chromatically facilitated motion perception. Our stimulus consists of alternating rows of innominate red and green dots that are displaced by half of their interval every second. At 1.5 Hz. Direction can only be disambiguated through color. Our human psychophysical data confirm that color facilitates motion and, furthermore, demonstrate that degree of facilitation varies with size of spatial displacement.

Neurons in cortical visual area MT are highly selective for direction of motion yet unselective for color. We recorded from single MT neurons in rhesus monkeys with innominate red/green pair was found for each cell by the minimal response elicited by a drifting green bar varying in luminance on a red background. The apparent motion stimulus was then placed in the cell's receptive field. Responses to chromatic correspondence were compared in the preferred and null directions. Although direction selectivity was commonly weaker than that elicited by luminance correspondence, significant color-facilitated motion correspondence was seen for the majority of cells. Degree of facilitation varied as a function of spatial displacement.

These results confirm the presence of a functional chromatic input to the magno pathway and imply true 'chromatic gating' of directional selectivity. Psychophysical experiments suggest that color may be but one of many low-level cues (e.g. orientation) that facilitate motion correspondence. It remains to be seen whether our facilitation is a general phenomenon underlying direction selectivity in MT.

503.14 DISTRIBUTION OF RESPONSE PROPERTIES ACROSS TOPOGRAPHIC SUBDIVISIONS OF MACAQUE PRELIMINAR GYRUS, M. Youakim and J.S. Balzer. Dept. of Physiology, University at Buffalo, SUNY, Buffalo, NY 14226.

We have studied response properties and topographic organization of the prelimentary gyrus in four hemispheres of two monkeys, behaving in a small-field macaque monkeys. Topography in the prelimentary macaque is similar to that reported for the rhesus (Maquire and Balzer, 1984). A vertical meridional representation (VMR) ran diagonally across the gyrus 10-13 mm lateral to the junction of the lunate and intraparietal sulci. Anterior and lateral to the VM was area 4 (Area AL of Maquire and Balzer). Posterior to the VM, two response properties, orientation sensitivity and background modulation, distinguished PM from V4. Area PM contained a significantly greater proportion of orientation sensitive cells (73%, 47/64) than V4 (35%, 30/86, p < .001). In V4, however, a greater proportion of cells (37%, 15/41) showed dramatically enhanced responses to a visual stimulus in the presence of a background pattern than in PM (58%, 120, p < .05). Supported by MH42130 and the Whitall Foundation.

503.15 DERIVATION OF POPULATION VECTORS FROM THE RESPONSE SPECIFICITIES OF VISUAL CORTICAL CELLS.
M.P. Young* and K. Tanaka. Lab. for Neural Information Processing, RIKEN, Wako, Saitama 351-01, Japan.

It has often been suggested that the cell population may be a useful level at which to analyse activity in neural systems. Population vectors have been derived for motor cortical cells by assuming that cell preferences may be defined in the vector space in which movements are made (Georgopoulos et al., Science, 233:1416-9, 1986). Population vectors could also be derived for visual cells with simple stimulus specificities by an analogous assumption concerning the sampling of the visual space. Many visual cells, however, have more complex 'trigger' features. The first step in the derivation of population vectors for these more complex cells is to find a Euclidean space in which the features of the cells may be represented. This can be accomplished by applying multidimensional scaling (Shapiro, R.N., Science, 210:390-398, 1980) to a table of the responses of cells to a number of stimuli. The stimulus preference for each cell may then be represented in the derived space as a vector, or, to preserve information about the relative strengths of the vector, it may be represented as a statistical distribution. Population vectors or population distributions can then be derived by summation after weighting the cells vectors by response magnitude.

This approach can be applied to the responses of local populations of anterior intertemporal (AI) and inferotemporal (IT) cell complex stimuli. A feature of these local populations is that neighboring cells have similar stimulus specificities (see Fujita, l, et al., this meeting). This population analysis demonstrates that visual selectivity is signalled by a particular local population of cells. Population distributions, derived in this way, allow comparison between the properties of cell populations and psychophysical representations of the discriminability of stimuli, when these are given in terms of signal detection theory.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
SENSORY SYSTEMS—VISUAL CORTEX: RESPONSE PROPERTIES

Sensory systems—visual cortex: response properties

502.17 Visual and oculomotor properties of single neurons in posterior cingulate cortex of rhesus monkey. S. Y. Minns, C. R. Olson, and M. E. Goldberg. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892, and Dept. of Psychology, George Mason University, Fairfax, VA 22030.

The posterior cingulate cortex (PCC) of the rhesus monkey is widely regarded as a paralimbic area whose functions related to emotion and motivation. However, it is linked by a strong reciprocal pathway to parietal Area 7a which participate in visual, attentional, and oculomotor processes. To see if CCG is involved in these processes we recorded from single neurons in CCG in a rhesus monkey trained to perform a variety of visually guided oculomotor tasks.

Neuronal activity was modulated in relation to task performance in roughly half of the neurons forming our sample. The factors most strongly influencing the level of activation were the position of the eye and the amplitude and direction of saccades. Neurons influenced by orbital position characteristically discharged tonically when the eye was in an extreme position and exhibited a graded decrease of discharge as the eye deviated from this position. Neurons influenced by saccades changed their firing level at or shortly after saccade onset. They were broadly tuned for direction and fired most strongly during and after large saccades. Although initial and final orbital position affected the response of these tonic sacadic neurons, they did not discharge when a seemingly effective saccade was acquired by an ineffective direction or amplitude of saccade. These neurons did not respond to small (∼5°) spots of light, but because even sacadic-related activity was often modulated by background illumination they may be visually responsive.

These results suggest that CCG neurons participate in visuomotor or visuospatial functions depending upon accurate knowledge of eye position and recent eye movements. They do not support a uniquely emotional or motivational function for CCG.

502.19 Retinotopic organization and receptive field characteristics of neurons in the visual Wulst of the pigeon. J. E. Sersole and R. J. Frost. Department of Psychology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

In the avian visual system, a significant number of retinofugal fibres project to the dorsal thalamic nucleus complex (n. oculi lateralis thalami, or OPT). The main ascending target of the OPT is a laminated structure on the dorsal surface of the telencephalon known as the Wulst. Although there are reports in the literature which indicate the presence of retinotopicy in the pigeon Wulst, the retinotopic organization of this structure has not been systematically investigated. We used standard electrophysiological techniques to record from single units in the pigeon Wulst, and mapped their receptive field positions. In addition, we measured the sharpness of tuning of units selective for the orientation of bar stimuli.

We mapped the contralateral visual field within the Wulst, and observed a heavy overrepresentation of the region. Binocular overlap, forty percent of the isolated units were orientation-selective and appeared to be more broadly tuned than neurons in homologous striate cortex. The data are consistent with previous suggestions that the visual Wulst may be functionally analogous to mammalian striate cortex.

503.1 Neurons in primate tongue motor cortex alter their firing rates during swallowing. G. M. Murray and B. J. Sessle. Faculties of Dentistry, Univ. of Toronto, Canada M5G 1G6, and Univ. of Sydney, Australia 2006.

Our recent studies have shown that many neurons in tongue motor cortex (tongue MI) alter their firing rates during a tongue-protrusion task performed by a monkey. This study's aim was to see if tongue MI neurons also alter their firing rates during semi-automated orofacial movements such as swallowing. Extracellular single neuron recordings were made from tongue MI (defined by intracortical microstimulation; <20 μA) in an awake monkey (M. fascicularis) swallowing the fruit juice reward associated with each successful performance of tongue-protrusion task trials; EM recordings were also made from the genioglossus muscle. For each of 76 tongue MI neurons, the firing rates during the task and during the period of genioglossus EM activation associated with the swallow were compared statistically (ANOVA) with that during a control period. The neurons were found to be (i) swallow-related only (14%, 11/76, i.e., each neuron exhibited significant changes (vs. control) in firing rates during swallowing on (ii) both swallow- and task-related (33%, 25/76), or (iii) unrelated to swallowing and the task (20%, 15/76). These data suggest that tongue MI neurons involved in the execution of orofacial movements associated with swallowing.

Supported by Canadian MRC.


In order to establish the modality specificity of AEC and to determine whether it is a polysensory area, extracellular recordings were carried out along the anterior ectosylvian gyrus (AES) in anesthetized-curarized cats. Three types of stimuli were used: visual stimuli consisted of light or black bars presented at optimal location and speed; air puffs and light touches were applied to different body regions made up the somatosensory stimuli; auditory stimuli were presented through a speaker positioned either in front or on either side of the animal. The responses were analyzed by studying the tuning of afferent responses to different types of stimuli and the number of significant changes that were present. The responses were then classified into primary, secondary, and tertiary categories.

The results showed that unimodal, bimodal, visual-auditory, visual-somatosensory, auditory-somatosensory, and trimodal units were distributed almost equally in the AES, with a slight predominance of the latter type of units. Thus, approximately 2/3 of the units responded to at least two modalities. The receptive fields (RFs) of cells with visual or/or somatosensory modality were almost always large and bilateral. The somatosensory RFs covered either the face, whiskers, head, or/and limbs and the tail. The response to stimulation in one modality in a multimodal unit was not always equal to that evoked in the same unit to stimulation of a different modality. There were no significant differences in the modality distribution along both the ventral and dorsal surfaces of the sulcus. The high proportion of multimodal units suggests that AEC is a polysensory area which is probably involved in integrating sensory input with action.
503.3 MODULATION OF RESPONSES OF PRIMATE FACIAL SOMATOSENSORY CORTEX SI NEURONS TO PERIPHERAL STIMULI DURING TRAINED MOTOR TASKS. L.-P. Lin, G.W. Murray, and B.J. Seagle. Fac. Dentistry, Univ. of Toronto, Toronto, Ont., M5G 1S6

Modulation of somatosensory transmission in SI neurons has been implicated in motor control. This study’s aim was to see if somatosensory transmission in the face SI neurons is modulated in relation to oralofacial movements. Extracellular single neuron recordings were made in awake monkeys (Macaca fascicularis) trained in tongue protrusion and biting tasks. Mechanical (200 ms) or electrical (100 μs) stimuli were applied to the mechanoreceptive cutaneous field (RF) of single neurons with a porcine RF, or the lingual nerve was electrically stimulated for neurons with a tongue RF. Evoked responses of neurons were tested during the biting task but was noted for 1-s prior to, during the movement associated with each task. Depression of evoked activity occurred in only 10 of 19 neurons tested during the biting task and was noted in all 16 neurons tested during the tongue task; modulation of the evoked activity often occurred 50-160 ms before the onset of tongue EMG activity associated with the tongue task. No modulation was noted prior to or during voluntary orofacial movements; the presence of modulation may depend on RF location and the nature of the task. Supported by Can. MRC.

503.5 MULTIPLE CONTROL OF CEREBRAL CORTEX IN EYE MOVEMENTS. Y. TAMAI and A. KIMURA, Dept. of Physiology, Wakayama Medical College, Wakayama, Japan

Neuron discharges in the eye movement-evoking cortices (EMECs) were studied in relation to visually or auditory guided saccades of the cat. Experiments were carried out using nonanesthetized cats with chronic microelectrodes implanted stereotaxically in the caudal medial wall of the hemisphere under the cruciate sulcus, the mediolateral and bank of the presylvian sulci, the fundus of the presylvian sulcus and the ventral bank of the anterior ectosylvian sulcus. The cat was sitting in the box and moving her eyes according to the light or sound signals. There were two types of neuron in the EMECs, the one was locked to the sensory signal and the other was locked to the eye movement. The movement-locked neurons, however, were not always active in an specific EMEC but discharged simultaneously or alternatively between the EMECs. These results suggest that the eye movement may be controlled by many cortices located in the different portion of the cerebral cortex.

503.7 DEFICIT IN UNIMODAL (TACTILE) AND CROSSMODAL DELAYED MATCHING FROM COOKING PREFRONTAL CORTEX. J.M. Foster, B.V. DiMattia*, K.A. Buyuk*, and W.W. Shinsky* Department of Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The purpose of this research was twofold: (A) to acquire normative data on the ability of monkeys to learn a haptic (tactile) memory task (delayed matching-to-sample); and (B) to examine the effects of reversible cryogenic lesions of dorsolateral prefrontal cortex on a haptic related task. A task-trial essentially consisted of the following sequence: (1) palpation or visualization of a stereomometric object (the sample); (2) delay (5-60 sec); and (3) presentation of two objects for a haptic or visual choice—rewarded if correct (i.e., if the chosen object matched the sample object). These monkeys were trained to perform the task with several different objects differing in size, shape, or texture. Learning rate and efficiency varied among animals. One animal did not learn to make haptic choices. The other two learned to perform the task faster and to a higher level in the visual mode (visual sample, haptic choice) than in the other two modes (haptic-visual and haptic-haptic). In all three animals, learning rate and final level of performance depended on the object pair and the delay. Performance decreased as a function of delay. After training, cooling probes were implanted—under general anesthesia—on dorsolateral prefrontal cortex and posterior parietal cortices in two animals that learned the task in all three modes (visual-haptic, visual-visual, and haptic-haptic). Bilateral prefrontal cooling to 15°C induced a reversible performance deficit in all three modes. No significant performance deficit was induced by bilateral parietal cooling (19°C) on haptic-haptic performance. The results indicate a deficit in taskual as well as visual short-term memory in relation to haptic modulation of frontal cortex. This deficit occurs for the three modes of cross-temporal integration performed by the animals in these experiments.


Single neuronal activities were recorded in the orofacial area of the first cortical somatosensory (facial SI) of 8 awake cats, to investigate the functional role of sensory information during mastication. Fifty-nine percent of the auditory inputs of facial SI (102/6-620) showed a rhythmic burst firing in relation to the movements of the jaw and the tongue during mastication. Approximately 75% of the orofacial neurons were found in the perioral and the tongue projection areas of the orofacial SI. These MR neurons had receptive fields in the perioral region and tongue and teeth were stimulated to be masticated during mastication. However, distinction between MR and non-MR neurons with the same receptive field depended on differences in the direction-sensitivity and threshold value to stimulation during mastication. These results suggest that the MR neurons would supply sensory informations required the performance of mastication. We investigated the neuronal activity in the motor cortex (MI) in relation to mastication. We will also discuss to be different in the neuronal activity between the SI and the MI.

503.6 DISTRIBUTION OF MOVEMENT RELATED POTENTIALS (MRP) IN A MONKEY: EFFECTS OF A UNILATERAL LESION OF THE SUPPLEMENTARY MOTOR CORTEX. N.S. Nicholson and I.C. Arenz. Albert Einstein College of Medicine, New York, USA.

Identification of the neural generators of MRPs requires topographic mapping of individual components. For this purpose, we placed 104 epidural electrodes extending bilaterally from the principal to the parietal sulci. The animal was trained to perform self-paced, right wrist movements; EMG was used to trigger MRPs averaged in 1-s with 500 ms pre-movement. Five MRP components were identified; 3 were mapped in detail. N2A and N2B are early components that each begin less than 325 ms before the EMG burst and peak at approximately 70 ms prior to, and coincident with, the EMG, respectively. These components are marked overlying ipsilateral regions. N2A is also distributed bilaterally, centered over MI and positioned ipsilaterally. It is characterized by a broad contralateral distribution centered over the somatosensory region.

Following initial mapping, the animal suffered an accidental puncture lesion of the left SMA, portions of the basal ganglia and the anterior commissure were also involved. Clinical sequelae included focal motor sequelae followed by temporary right upper extremity paralysis. After functional recovery the contralateral EMG was indistinguishable from pre-injury studies. N2A, however, was significantly reduced in amplitude (P<0.01) and its distribution was restricted to the posterior contralateral MI. N2B showed similar but less severe changes (approximately 65% decrease contralaterally). P2B showed little overall change in amplitude or distribution.

These findings suggest that SMA participates bilaterally in the initiation of movement as repressed by the phasic negativities seen immediately prior to and coincident with the EMG burst. Furthermore, the P2 in SMA participates in the initiation of voluntary movement. Further studies are required to elucidate the mechanisms of this interaction.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

The directional saccade activity of prefrontal neurons was thought to reflect a mnemonic event, holding "on" information that will guide the correct response at the end of the delay. In the present study we hoped to learn more about the nature of the mnemonic code which the neuron uses, which it reflects information about the behavioral context or the direction of the impending response. To examine this issue, we compared neuronal activity under two task conditions: a delayed saccade task (DS), in which the correct saccade was always in the same direction signaled by the cue, and a delayed anti-saccade task (DAS), in which the required saccade was in the direction opposite to that signaled by the cue. A total of 44 neurons with directional delay-period activity in the DS task was examined in the DAS task. The majority (n=30) showed the same directional delay-period activity, i.e., responded only when the cue was present at the same location in both tasks, regardless of the direction of the saccade. The delay-period activity in these neurons was further characterized by their pattern of activity on DS trials in which the monkey failed to inhibit the proposed response. On these trials, which are associated with correct responses on DS task, the neuron also discharged in accord with the location of the cue. Ten additional neurons showed delay-period activity which depended on the direction of the forthcoming saccade and the remaining 4 neurons exhibited directional delay-period activity only in the DS task.

The present results with respect to saccule motor responses are in accord with data originally obtained for manual delayed-response tasks by Niki and Wanamaker (Brain Res. 105:79, 76). Both studies indicate that independent prefrontal neuronal code and response direction and that such is a distinct component of the circuitry involved in memory-guided responding. We speculate that the smaller proportion of neurons that code saccade direction are less closely structured which generate saccades, whereas the larger proportion of neurons that maintain a representation of sensory events could supply directional information to be read out by any of several motor centers. The independence of sensory and motor neuron suggests that the prefrontal cortex provides a mechanism for flexible response choice. Supported by M144866, MH138546.


Recently we proposed a new model that treats the cerebellum as an array or network of interconnected modules that function as an adaptive pattern generator (Bouc et al, Neural Networks for Control, Miller, Sutton & Werbos, 1990). Here we report on the capabilities of small arrays of these modules for learning to control two-dimensional movements. The present model is based on the cerebellum and the motor cortex and the goal was to control a set of planar limb movements in a task analogous to the one used by Georgopoulos and coworkers to study populations of neurons coding movement directions. The four neurons between parallel fibers and Purkinje cells (PCs) were adjusted using training signals transmitted through fiber inputs and a training rule inspired by the biochemistry of intracellular messengers regulating LDL at parallel fiber synapses. Changes were assumed to be due to fibrous corrective movements with a probability that was broadly tuned to the direction of the correction. Retrieval of a motor program from memory was controlled by a preselection process that turned PCs on and off in a bistable manner. The motor program was then initiated by the initially summed propositional and input cues caused PC transitions to their on-states, whereupon strong inhibition terminated the program. The movement trajectories were qualitatively similar to those observed experimentally. (Supported by ONR N00014-88-K-0339.)

503.10 TOPOGRAPHIC REPRESENTATION OF MEMORY IN THE PREFRONTAL CORTEX OF MONKEYS REVEALED BY LOCAL APPLICATION OF BIACUCILLINE. P.E. Goldman-Rakic, Dept. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

To examine the functional role of the prefrontal cortex for spatial working memory, bicuculline was locally injected into the dorsolateral prefrontal cortex while monkeys performed a delayed response task to items that were not previously associated with a particular cue. The monkeys fixated a central spot and made memory-guided saccades to a visuallay available target to recall faces. This led to a depression of the delayed period prior to a brief (usually 5 sec) delay period. In each condition, the target word (n=30) was presented > 2 peripheral locations with different eccentricities and/or directions. The local injection of bicuculline (1-10 mg/ml) caused deficits in the task to a variable degree in each condition. The deficit was sensitive to the duration of delay and longer delays were associated with larger deficits. Furthermore, the relationship between the affected location of target and injection sites showed a topographic manner. Neuronal responses were affected by injections into more lateral regions, and upper locations were affected by injections into more medial regions of the dorsal prefrontal cortex. No deficits appeared in a control task given in the same session which required only sensory-guided saccades. These results suggest that memory modules for specific visuospatial representations are topographically represented on the prefrontal cortex.

503.12 COMPUTER STUDIES OF THE ROLE OF NMDA RECEPTORS AND POSITIVE FEEDBACK LOOPS IN THE GENERATION OF DESCENDING MOTOR COMMANDS. L. N. Eisenman, J. Keifer and J.C. Houk. Dept. of Physiology, Northwestern University, Chicago, IL 60611.

Positive feedback in recurrent loops between the cerebellum, red nucleus and motor cortex is thought to be important in the generation of central motor commands (J.C. Houk, Models of Brain Function, p. 339, 1989). A computer simulation showed how the cerebellum elements had been implemented to test this theory. Positive feedback between reciprocally connected neurons was triggered by a transient sensory input and regulated by preset levels of inhibitory input from cerebellar Purkinje cells. The activation function for a neuron was modeled by physiological data derived from the in vivo vittu preparatory target which had been presented in the red nucleus are produced by recurrent excitation from the cerebellar circuitry mediated by combined NMDA and non-NMDA-dependent synaptic transmission. The voltage dependence of the NMDA channel, however, was a threshold phenomenon. The cerebellar sites in the activation function assumed for the model neurons. When a pair of cells with these properties were reciprocally connected, two stable regions emerged. There was an off state and an on state that had a wide range of stable operating points. The intensity of the input in the on state could be modulated by inhibitory input reflecting the effect of Purkinje cells. In contrast, when activation functions without discontinuities were used, the on state of the network was much less conceivable. Twelve reciprocally connected two-cell pairs were interconnected to model divergence in linkages between the motor cortex and the interpositus nucleus of the cerebellum. Each of the 12 elements is considered to have a preferred direction sensitivity. With this network several observations of Georgopoulos and colleagues regarding population vectors in motor cortex were reproduced. The values of the inhibitory input determined the final state of the network which reproduced activity patterns that resembled population vectors. It was further possible to simulate rotations of population vectors prior to changes which are related to neural rotation of images (Georgopoulos et al., Science 243, 324, 1989).
503.15 TEMPORAL PATTERNS IN A CAM. H. Stowell. RRIB Lab., 120 Nature Creek, Millyedgeville CA 91061 USA.

How does a Content Addressable Memory (CAM) store and recall the timing of sensory stimuli? Continuing EEG data from humans [1] imply that quasi-limit-cyclic activity in the 'theta-alpha' range (3-13 Hz) is critical to working memory in rabbits. Five native subjects, trained to minimize extracranial artifacts, show phase constraints and phase ordering at above-chance level in scalp-conducted, vertex-to-earlobe EEG when attending to novel sequences of auditory stimuli; this phase ordering appears in both stimulated and silent trials, for small averages and single epochs. In (10) when subjects have a knowledge of self-paced time-frame, but only at chance level within an unknowable time-frame. Momentary drowning, with failure to recall, results from the lack of a suprathreshold and always detectable rhythmic pattern, shown abrupt EEG frequency slowing from 10 to 3 Hz before the stimulus time-window and a much larger and later 'vertex evoked potential'. The wakeful effects tend to disappear with subjective familiarity over weekly sessions of 1-5 hrs.


An IC chip has been designed and fabricated by MOSIS for alternately recording from or stimulating each of 16 electrodes placed in the CNS. The chip integrates 16 stimulatory and 16 recording channels. Each stimulus or recording channel is connected to an on-chip memory register that controls the channel's output. Each stimulatory channel has an internal timing circuit, which is used to stimulate one or more of 16 sensor wires for stimulation, or all inputs can be employed as high impedance sensors. As presently configured, the chip is mounted in a 1 inch square by 1 mm thick leadless chip carrier (LCC) which in turn is wired to a small LCC socket. It is then wired to five 12-pin, 2 mm thick Microtech connectors which allow the unit to be mounted directly to a female Microtech headstage. A testing program has demonstrated successful recording from typical neurons detected by multiple 25 micron microwires implanted in neonatalum of awake behaving rats. The stimulation circuitry also has been successfully tested. The practical dynamic range of bipolar stimuli is +1.2V to -3.8V but may be extended. These results are in use for the design and development of the next generation chip which will include 32 IO channels, but with reduced IO lines by means of multiplexing and serial control. Target installations will include embedding of the chip in configurations employing multichannel silicon probes with microwire interconnections. The latter are under development with Dr. S. Ang at U. Arkansas. Supported by the Communities Foundation of Dallas, MH44337, AFOSR 90-0146, DA02338, and the Biological Humanities Foundation.

503.17 DYNAMIC CONVERGENCE IN NEURAL ASSEMBLIES. P.H. Bedenbaugh1, G.I. Gestaut2, A.M.B.J. Antwer3, 1 Departments of Physiology and Biophysics, University of Pennsylvania, Philadelphia, 2 MPI for Biological Cybernetics, Tübingen, FRG.

When interpreting the simultaneously recorded activity of several neurons one must remember that the neurons are embedded in a large, densely interconnected network. The firing of the observed neurons is partly determined by the activity of the many unobserved neurons. For any two observed neurons, the presynaptic network may be subdivided into a shared pool that projects to both cells and a pool that projects only to one cell. Let us call the influence that a presynaptic pool, shared or unshared, exerts on an observed target neuron the 'dynamic convergence'. In contrast to the static, anatomical notion of a converging projection, dynamic convergence is a physiological measure that reflects rapid shifts in the active and inactive parts of neural structures. Recent theoretical work has shown that the properties of this dynamic convergence, both its magnitude and its internal correlation, dramatically affect the strength of correlation between the activities of the observed presynaptic neurons.

It is intended to directly measure the activities and relative influences of the various presynaptic pools. We can learn something about the unobserved presynaptic pools, and hence the dynamic convergence by studying the joint statistics of the observed spike trains. We are investigating a dynamic generalization of the coherence function. The coherence function is the cross spectrum of two processes normalized by the product of the square roots of their individual auto-spectra. Coherence is widely used in geophysics and EEG-analysis to discern the extent to which two spatially distinct observations are driven by the same underlying process. For linear systems, coherence varies between zero and one and provides a static measure of shared input.

This type of quantitative measure is relevant for evaluating contrasting ideas of neuronal assembly organization. Each neuron in a Helix aspersa assembly participates in several loosely coupled networks. Input activity imposes a particular organization by igniting one such network. Other models are built on tightly coupled and essentially disjoint groups of neurons whose organization would appear invariant with stimulus conditions. Dynamic convergence would be highly stimulus related in the first case, and not so in the second.

Supported by ONR N00014-90-0769.


The flatworms (phylum Platyhelminthes) are the first phylogenetic group to evolve 'true brains' and thus, are appropriate models for the study of the original functions of the brain. Hymenolepis diminuta, a parasitic flatworm, is capable of coordinating locomotory activity that is not affected by the removal of its brain. This parasite uses a complex adaptive behavior, consisting of retrogade peristaltic waveforms (PL) to maintain its position in the small intestine. In addition, it also uses this behavior to migrate anteriorly in the intestine (in response to cues elaborated during host feeding) to position the strobilus in the regions of highest nutrient concentrations. PL frequency varies along the strobilus in a coordinated manner, occurring at a rate of 23.5 ± 1.1 Hz in the cephalic region and 4.3 ± 1.3 Hz in the caudal regions. The form and frequency of this complex behavior along the strobilus is not significantly altered by denervation, but positions in strobilus to a thermal gradient (22-32°C), intact worms use PL waves to migrate uphill to a preferred temperature of ~30°C (thermo-orthokinesis). Cessate worms also move similarly up the gradient, but there are some qualitative differences in the behavior. Although worm survival in the host requires the brain, it is concluded that the elaboration of PL waves and the coordination of these movements along the body is under the control of the peripheral system.

504.2 TEMPERATURE DEPENDENCY OF WING-BEAT FREQUENCY IN INTACT AND DEAFFERENTED LOCUSTS. J.A. Foster and R.M. Roberts. Department of Biological Sciences, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

Locusts do not regulate thoracic temperature during flight. We show that the thoracic temperature of a flying locust can exceed ambient by as much as 10°C. In vitro, thoracic temperature has been shown to affect wing-beat frequency in intact Locusta migratoria. This study investigated the temperature dependency of wing-beat frequency in intact and deafferented locusts.

Intact tethered locusts were flown in a wind tunnel at different ambient temperatures for up to 3 hr. The effects of these high ambient temperatures were monitored. EMG recordings were made in a deafferented locust perfused with different temperature saline. Wing-beat frequency was shown to increase and decreasing linearly to temperature in both the intact and the deaffeted situation. The slope of the rise in wing-beat frequency with experimental increases in thoracic temperature was similar in intact and deafferented animals. These results demonstrate that there is a central effect of temperature on wing-beat frequency. Further intracellular experiments are necessary to investigate the neural basis of these temperature effects.

INVERTEBRATE MOTOR FUNCTION

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
504.3
EFFECTS OF TEMPERATURE ON PROPERTIES AND INTERACTIONS OF FLIGHT NEURONS IN THE LOCUST. R.M. Robertson and J.A. Ratnie. Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

In an increase in the thoracic temperature of intact Locusta migratoria tethered flight results in an increase in wingbeat frequency. Similarly, increasing the temperature of the thoracic ganglia in a deafened preparation results in an increase in the frequency of the rhythmically generated flight rhythm. We investigated the neural bases for these changes using a deafened preparation of Locusta migratoria. Intracellular recordings were made from the neuropile of identified flight neurons using standard techniques. The temperature of the ganglia was altered by superfusing them with warmed saline (25-40°C).

To date we have found the following effects which are consistent with previous studies of temperature effects on insect neurons. The duration and amplitude of action potentials was decreased. The latency of direct synaptic connections was approximately halved by a 10°C rise in temperature. In contrast with previous reports of an increase in the amplitude of excitatory postsynaptic potentials (epspS) we found that the amplitude of inhibitory mps was decreased. Preliminary evidence indicated that this reduction may be due to membrane hyperpolarization brought on by increased temperature.

504.4
PLASTICITY IN THE FLIGHT SYSTEM OF THE LOCUST. A. Bischeher and K.G. Pearson, Dept. of Physiology, University of Alberta, Edmonton, Canada.

The initial depolarization in elevator motoneurons in intact flying locusts is induced by the phasic activity of the hindwing tegulae during the downstroke of the wings. Previous results have shown that removal of the tegulae results in a decrease in the rate of depolarization in elevator motoneurons. This decrease in rate is not due to a decrease in the amplitude of the tegulae's phasic activity. We have now quantified these effects and found that they are not significant. The average wingbeat frequency (WBF) of intact flying locusts was about 20 Hz and the average D-E intervals were about 20 ms for the forewing and 25 ms for the hindwing. Removal of the tegulae decreased the WBF and increased the D-E intervals. The initial value and caused an increase in the D-E interval of about 12% (average decrease 14% D-E interval forewing: 12 ms; average increase D-E interval hindwing: 17 ms). The increase in the D-E interval in the elevator motoneurons disappeared. A period of about 2 weeks following the removal of the tegulae there was a progressive increase in the WBF and about 90% of its value) and a progressive reduction in the D-E intervals. 14 days after the removal of the tegulae the average D-E interval for the forewing was about 20 ms and the average D-E interval for the hindwing was about 28 ms. Intracellular recordings from the elevator motoneurons in recovered animals during flight showed that the initial depolarization was comparable to that occurring in normal intact animals. The neural mechanisms underlying the recovery of the flight pattern following tegula removal are now being investigated. Preliminary data have indicated that there are changes in the characteristics of the central rhythm generating network as well as alterations in the influence from other wing proprioceptors in establishing the motor pattern.

504.5

In the abdominal and thoracic ganglia of the locust central nervous system several populations of midline cells with bilateral axons exist, that release neuropeptide substances. In the abdominal ganglia some of the cells could be identified with respect to their target regions. In the thoracic ganglia, among the cells with bilaterally symmetrical axons are two "classical" DUM (dorsal unpaired median) cells that innervate all skeletal muscles of the abdomen and stain with an Octopamine antibody (M.Eckert, Jena). Two other cells with bilateral symmetrical axons, also resembling DUM-cells, run exclusively to the insect heart. One of the two cells stains with a FMRFamide-like antibody. Neurons with bilaterally symmetrical axons exclusively run to the heart and do not stain with either an Octopamine or FMRFamide-like antibody.

504.6
MEMBRANE PROPERTIES OF LOCUST NONSPIKING LOCAL INTERNEURONES. G. Liberman, California Institute of Technology, Biology Division, 156-29, Pasadena CA 91125.

The electronic and active membrane properties of nonspiking local interneurons were studied, using the switched current- and voltage-clamp techniques in neuropilar recordings. The average transmembrane potential (Vt) of the interneurons was 52.6 ± 8.5 mV and the input resistance (in the linear region of the I-V curve) was 16.5 ± 2.4 MΩ (n = 33). The average synaptic current evoked by a 150 ms depolarizing current injection to 32 mV was 2.35 ± 0.64 nA, which corresponds to an average synaptic conductance of 1.5 ± 0.4 nS. The average synaptic current was accompanied by a rectification of the membrane, which was most easily observed for the responses to depolarizing current injections. The rectification was generally activated from potentials more negative than Vt, and was accompanied by a decrease in input resistance and membrane time constant. The resting membrane for example had a time constant of 26.4 ± 8 ms (n = 3). This outward rectification was due to the least 2 conductances with different inactivation kinetics, similar to the A- and B-type delays in their conductances. This inward rectification was observed upon injection of hyperpolarizing current. In about 60% of the recordings, an active and ITX-resistant depolarizing process could be evoked by rapid depolarization around Vt. The voltage-dependent properties of the membrane of the nonspiking interneurons had dramatic effects on the time course of natural or evoked unitary PSPs. The half width of EPSPs, for example, decreased by a factor of 7.5 if the membrane potential was shunted from -90 to -60 mV. Supported by The Royal Society, SERC (UK), and the Hasselblad Foundation.

504.7
DISTRIBUTION OF STRAIGHT INPUT OF WING STRETCH RECEPTORS IN DENDRITIS OF LOCUST FLIGHT MUSCLES. H. Schneider, Universität Konstanz, Biologie, 7750 Konstanz, FRG.

To study the integrative properties of identified motoneurons (MSs) synaptic input was compared in various cell structures using double intracellular recordings. Overlap of the arborizations of the pre- and postsynaptic neurons was reconstructed by different intracellular staining (DIS) with Lucifer Yellow and Texas Red.

The morphology of the 2 sublar MSs (SAMMs, wing depolarizer) in the somata of the locust Locusta migratoria is characterized by 6 primary dendrites (PD) which originate laterally and medially from a neuropilar segment (NPS). Both SAMMs, EDPs and EPSPs are directly elicited by activity of forewing (msSR) and hindwing stretch receptors (nSR). These EPSPs were recorded with no latency shifts and with time course in the PDs and the intracellular input resistance of the PDs. However, there appear to be different input resistances in the medial PDs and spread intracellularly to the lateral PDs. DIS of MSs and SAMMs support these intracellular measurements. The MS input is well all recorded PDs; the mosaic, and SK-2 was the major synaptic input. These results suggest that a functional differentiation of PDs of SAMMs with regard to synaptic input from SKs does not occur as early as in the thoracic ganglia. There is direct physioloical connections and the electronic spread of EPSPs. Supported by DFG (Kn 240/14-1,2).

504.8
IN FLIES, MOTOR NEURONS SUPPLYING INDIRECT (POWER) AND DIRECT (STEERING) FLIGHT MUSCLES OCCUPY DISCRETE NEUROPSI SUPPLIED BY CHARACTERISTIC MOTION-SENSITIVE DISCORISING NEURONS. Nicholas J. Strassman and Carol Askart.

Antar Research Lab., Div of Neurobiology, Univ of Arizona, Tucson, AZ 85721.

Specific flight muscles [maxillae, Wieser; Nachigall (1983) Zoolomorphology 108:169-182] have been impaled with carbon electrodes to recordically fill motor neurons supplying them. Silver-intensified neurons were reconstructed, and related to specific degree and within thoracic ganglia. Two sets of functionally similar muscles (DLMs and DVMs) and one set of non-floral muscles [hgt, 2 pro- and supinators. Functions: Wieser and Nachigall (1983); Hecht (1960) Z Vergl Physiol 54:416-460] are supplied by superficially phasoreceptor motor neurons whose bilateral symmetrical dendrites are visible at bilateral terminations of descending neurons (DNs) responding to symmetrical movements back and forth at the vertex of the fly's abdomen. These neurons are visible at terminations of DNs that respond to asymmetrical shifts of the visual panorama, or to small-field motion. Laterally and ventrally, unilateral motor neurons include those involved in vision and wing-vault control. Certain steering motor neuron (B-1, 3, 110) show cobra-coiling to specific functionally identified DNs and to primary sensory afferents from the wings and halteres, thus providing evidence for the convergent role of mechanosensory and visual inputs in flight maneuvers. Supported by NHI Grant No. R01 EY0 7151-01 and NSF Biological Centers Grant DBR 82-02002.
404.11 MUTATIONS AFFECTING K AND Na CURRENTS IN DROSOPHILA ALTER PHYSIOLOGICAL, PROPERTIES AND PRODUCE ABNORMAL SPONTANEOUS ACTIVITY IN THE GIANT T hypocrisy NEUROPATHY. J.L. Sheng and C.F. Wu. Dept. of Biology, University of Iowa, Iowa City, IA 52242.

The neural circuitry controlling the escape reflex in flies has been well characterized. We examined the physiological and behavioral effects of perturbing this pathway with the mutations Shaker (Sh), Hyperkinetic (Hk), and ether-a-go-go (egg), which reduce K currents, and a no action potential (nap), which reduces Na current. By recording from the dorsal longitudinal (DLM) flight muscles and the tergosternal (TTM) leg extensor muscles, we monitored spike activity underlying a jump-and-flight escape response to electrically-mediated, or by the flight pathway.

The mutants Sh, Hk, nap, and Hk egg, which affect K currents, have a longer refractory period for both DLM and TTM response, with latency unchanged. In Sh flies, the TTT shows longer latency and refractory period, but only in the TTM. Apparently the TTM pathway is preferentially affected because of differential expression of the nap gene or a higher sensitivities in this muscle.

Double mutant Sh nap flies show abnormal spontaneous rhythmic spiking in DLM muscles. This is not associated with wing beating, nor does it respond to tactile stimuli that turn flight on and off. The non-spike activity is seen in eg or Sh alone and are suppressed by nap in eg Sh; nap triple mutants. Simultaneous recordings from pairs of muscle dusters fibers indicate that the spontaneous activity is neurogenic and may involve the flight pattern generator or an interneuron mediating the giant fiber response. In Hk egg, rhythmic spiking is seen in the TTM in addition to the DLM, implicating the giant fiber, which innervates both muscles.


During insect metamorphosis there are dramatic changes in behavior that require alterations in neural circuits. Toward understanding this reorganisation, we have characterized the interactions between the bilaterally-paired abdominal stretch receptors (SRs) and identified motoneurons (MN) in the larval and adult stages of Manduca. The SR was left attached to the isolated nervous system and stimulated electrically. MNs were monitored intracellularly and identified by Lucifer-yellow injection. Our initial studies have focused on the intersegmental muscle (ISM) MNs which persist through development and show relatively little disability after conversion to the paramecium form. In both the larva and pharate adult, ISM MNs with bilateral dendrites receive excitatory input from ipsi. and contra. SRs in the same segment as the target muscle. Signal integration and treatment with tetanus suggest that these connections have a direct component. ISM MNs with unilaterals dendrites receive direct excitatory input from only the ipsi. SRs in both stages. The input to these unilaterals MNs for the differences is polysynaptic in both stages, but is inhibited in the larva and changes to excitatory during adult development. Similar, but weaker and more variable, connections were observed for MNs innervating the next posterior segments. All of these connections are consistent with opportunities for the SR terminals and MN dendrites to overlap at the light level. Interneurons which receive direct input from the SR have been identified. These may be involved in the polysynaptic connections described.

Thus, significant changes in the proprioceptive information supplied to the ISM MNs occur during metamorphosis. These changes may be related to the changes in abdominal posture and movements seen during this period.

404.13 MODELLING THE CONTROL OF INSECT SKELETAL MUSCLE. Jim H. Belanger, Dept. of Zoology, Univ. of Toronto, Toronto, Canada M5S 1A1.

Vertebrates and invertebrates use fundamentally different strategies for muscular control. Vertebrates use many motor units, with very little modulation of individual units, while invertebrates use very few units (often only one or two) combined with extensive peripheral modulation. This research seeks the extant modulating work on vertebrate muscle inappropriate for application to insects. I have attempted to develop a relatively low-level model which takes into account the extensive modulatory input to the skeletal muscle.

The model is a numerical simulation written in C and run on an IRIS workstation. The essence of the model is the production of graded contraction and the progressive recruitment of radially-oriented Ca2+ release sites. Potential changes at the surface of the model fibre passively propagate inward along the imaged (vobules, triggering local release of Ca2+). When the excitation contraction threshold is reached. Several sites within the model lobic for modulation. The most powerful effect is produced by lowering the excitation contraction coupling threshold, which can produce contraction in the absence of depolarization (as is seen, for instance in the action of the eptamele pantroptocin procluouc in many systems (Orchard, Belanger and Lange, J. Neurobiol. 20, 1989)). Other available modulatory mechanisms are ligand-gated calcium channels in the sarcoplasmic reticulum (SR), which are in parallel with the voltage-gated channel, and the Ca2+ uptake system of the SR.

By varying the nature of the synaptic input, this model allows an examination of the nonlinear interactions between the conventional (excitatory and inhibitory) transmitters and the unconventional transmitters. In addition, it allows quantitative examination of the hypothesis that co-transmitters are used for energy efficiency in motor control.

404.14 GABAERGIC PSESYI NAPTIVE IONIZATION AS MODULATOR OF MONOSYSNOPIFIC REFL EX IN CRUSTACEA. A. El Calarac, B. Esfahani, D. Cattaret, & J. El Calarac. CNRS INP, 31 Chemin Joseph Aiguier BP 91, 13402 Marseille Cedex 9 FRANCE.

In the thoracic crayfish in vitro preparation, it is possible to record intracellularly sensory terminals from a complex leg proprioceptor (the chordotonal CB) and motoneurons (MN) controlling the joint where this receptor is inserted. Mechanical or electrical stimulation applied to the CB receptor induces monosynaptic reflex resistance in MNs. By recording from CB terminals, we have demonstrated the presence of primary afferent depolarizations (PADs). In tonic preparation PADs occur continuously, but by contrast during phasic locomotion they occur at fixed time in the locomotor cycle. Spontaneous PADs were suppressed by bath application of GABA antagonists (Picrotoxin and Bicuculline). This result suggests that GABA acting on CB terminals of a CB reflex is modified. It was verified since small quantities of GABA or Muscimol applied near the recording site over the ganglion elicited a transient depolarisation of the impaled sensory terminal; they were coincident with a reduction of input resistance. The inhibitory role of GABA on the monosynaptic reflex has been confirmed since all MNs showed a marked and reversible reduction in compound EPSP amplitude when the CB nerve was stimulated during GABA application.

In Aplysia californica the peptide FMRFAs is synthetized by several buccal neurons (loyd et al., 1987) and has physiological actions in the feeding system. However, the identity and targets of neurons synthesizing FMRFAs is unknown. We are using intracellular lucifer yellow injections combined with immunocytochemistry to uniquely identify buccal motorneurons expressing FMRFAs-like immunoreactivity (FMRFALI).

Large ventral cluster cells of the buccal ganglia from 150 to 300 gram animals were identified by cell body size and position, axonal projections, and muscle innervation. Following ionophoretic injection of these cells with lucifer yellow, the ganglia were prepared as either 10 micron paraffin sections or whole mounts. Preliminary double-label experiments show that the motorneuron B3 expresses FMRFa-LI and projects to the ipsilateral buccal muscle 1/3. Future experiments will identify the remaining motorneurons expressing FMRFa-LI and test the properties of their neuromuscular synapses. Supported by NIH grant NS24662.


Ingestive behaviors involve coordinated head, lip, and buccal muscle movements. Buccal muscle movements are controlled by motor neurons and a central pattern generator (CPG) located in the buccal ganglion (BGC). In order to study the integration of lip and head movements, we identified lip motor neurons (C11 and C12) that have projections into the cerebral ganglia (CGB) and arborize in the ipsilateral buccal ganglion (LBG). These cells receive direct synaptic inputs from C2 and C3, histaminergic magnocellular neurons adjacent to the buccal cavity, and C10 that innervate the interneurons C11 and C12. After 3 days of injury, C11 and C12 neurons were found to be preceded by 15-20 microns. Current recordings showed that C11 firing increased in amplitude and frequency, leading to rhythmic lip movements during feeding by free-moving animals, whereas lip movement was not observed in the absence of C11 firing. These results support the idea that C11 and C12 neurons play a crucial role in the control of buccal movements in Aplysia.
504.21
PERIPHERAL MODULATION OF SWIMMING SPEED IN A PTEROPOD
MOLLUSC. R. A. Satterlie. Department of Zoology, Arizona State University, Tempe, AZ 85287-1501.
Three swimming speeds have been described for the pteropod mollusc *Clione limacina*: slow, fast and escape. The change from slow to fast swimming can be attributed to changes in the configuration of the pattern generator, and the recruitment of fast-twitch motor units, as opposed to the activation of only slow-twitch motor units during slow swimming. Escape swimming appears to involve activity in two sets of neurons that modify the activity of the swim musculature. In both sets of neurons, electrical activity is independent of pattern generator activity. One set of neurons monosynaptically excites both slow- and fast-twitch muscle cells and produces strong wing contractions which slow serotonin-immunoreactivity and produce a short-term excitatory modulation of muscle contractility. The time courses of activity in these two groups of neurons suggest that the motor neurons may be used for the initiation of escape swimming (startle response) while the modulatory neurons may be used for maintenance of escape swimming.

505.1 HUMAN CAUDATE AND PUTAMEN CHEMOARCHITECTURE
N. Selden, C. Geula, and M-M. Mesulam, Harvard U., Boston, MA.
Human striatal neurons containing the peptides choline acetyltransferase (ChAT), somatostatin (SOM) and calcium-binding protein (calbindin D28k) were visualized immunohistochemically using specific antibodies (generously provided by L. Hersh, R. Benoit and M. Celia, respectively).
Each peptide was associated with a different neuronal population. CHAT-positive neurons were relatively large (diameter: 25-35μ), multipolar in shape, intensely stained and exhibited no major variations in density between various components of the striatum. SOM-positive neurons were also darkly stained, but were smaller (15-20μ) and variable in pericellular morphology. The density of SOM neurons was higher in the caudate nucleus than in the putamen. Two populations of calbindin-positive neurons were observed: (1) a large population of lightly stained, small neurons (10-15μ), distributed throughout the striatum, with the greatest density over the dorso-medial caudate nucleus, (2) a small population of larger (10-20μ), darkly stained neurons, most frequently encountered in the lateral putamen. Approximately half of the latter neurons also stained positively for NADPH-diaphorase, an enzyme which is co-localized with somatostatin in striatal neurons. There may thus be an overlap between some of the somatostatin-positive and larger calbindin-positive neurons in the putamen. These chemorachdtic differences may be related to well-known differences of connectivity and behavioral affiliation between the caudate nucleus and putamen.

505.3 EFFECTS OF PRENATAL METHYLAMINOMETHANOL (MAM)
ADMINISTRATION ON STRIATAL PATCH FORMATION.
The formation of striatal patches, consisting of clusters of neurons and overlapping patches of nigrostriatal dopamine (DA) afferents, occurs prenatally. Persistence of the cellular patches after the prenatal removal of DA innervation (Snyder-Keller, Neurosci. Abst., 15:906, 1989), suggests that intact DA axons guide the association of patch neurons. Striatal patch neurons are born earlier (E13-15 in rat) than neurons of the matrix (E15-16) (van der Kooy & Fishell, Brain Res. 402:166, 1987), and this fact was used to selectively delete striatal patch neurons. The antiinhibitory agent MAM, delivered as a single dose (20-30 mg/kg) or two doses (total <45 mg/kg) on embryonic days 12-14, resulted in a reduction in forebrain mass and distortion of the striatum. Striatal cell counts, characterized by the substance P-dense and calbindin-poor, were still apparent, but consisted of a smaller number of relatively larger patches. These same neuronal patches could be selectively labelled by an injection of Fluoro-Gold into the neonatal striatum. The DA innervated arm, though less distinctive patchy during the first postnatal week, but ACHE-positive neurone assumed a fairly normal distribution. Thus, patches form despite large loss of striatal patch precursors, but the birthdate of the neurons remaining in patches has yet to be determined. (Supported by Tourette Syndrome Association.)

505.2 IMMUNOSTAINING FOR PROTEIN 10 CALCIUM-BINDING PROTEIN
FORMS STRIATOSOM-RELATED PATCHWORK IN THE RAT
STRIATUM. B. Quinn, A.M. Graybiel, L. Winkle, and D.M. Jacobowitz.
Brace Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, and Lab. of Clinical Science, NIMH, Bethesda, MD 20892.
Protein 10 (Pr10) is a newly described calcium-binding protein that is distinct from calbindin 28kD and that may be the mammalian homologue of calreinin (Winkle et al., PNAS 86:10139, 1989). We have studied the distribution of Pr10 immunostaining in the striatum of adult rat, and have compared patterns of Pr10 immunoreactivity to those of calbindin 28kD (CaBP)-like immunoreactivity, a known marker for a large fraction of the nigrostriatal dopaminergic neurons. Immunoreactive Pr10 was present in scattered striatal neurones. Neuront immunostaining for Pr10 was concentrated in patches (ca. 100 μm wide) with angular profiles scattered through the caudoputamen, and in the caudoputamen's dorsolateral rim. The most darkly stained Pr10-positive patches were in the dorsolateral part of the striatum, where CaBP immunostaining is minimal. Elsewhere, scattered Pr10-immunoreactive patches appeared to correspond to CaBP-poor zones (patches, striosomes). There was no clear relationship between the locations of the Pr10-positive patches and the Pr1D-positive neurones. Heterogeneous distributions of Pr1D-positive neurone also appeared in the nucleus accumbens-ventral striatum. These findings indicate that Pr10 calcium binding protein and calbindin 28kD have different and at least partly complementary distributions within the striosomes and matrix of the striatum. We thank the National Parkinson Foundation & NIH NS 25529, and P.C. Emson for anti-CaBP antiserum.

505.4 VITAMIN D-DEPENDENT CALCIUM-BINDING PROTEIN
(CALBINDIN-D-28K) IMMUNOREACTIVE NEURONS IN THE MONKEY
Department of Anatomy and Neurobiology, The University of Tennessee, Memphis, College of Medicine, Memphis, TN 38163.
A recent study has shown that many cholinergic neurons in the human nucleus basalis of Meynert (NBM) are immunoreactive for calbindin-D-28k (CaBP), a vitamin D-dependent calcium binding protein (Echternach et al., Brain Res. 286:499-506). However, the relationship of the CaBP-immunoreactive neurons with the cholinergic NBM neurons in other species has remained unclear. In this study, a mouse monoclonal antibody raised against CaBP (gift from Dr. M.R. Celio) and a rabbit antiserum raised against human placental choline acetyltransferase (ChAT) (Chemicon Inc.) were used in double-labeling immunofluorescence reactions to compare the distribution of CaBP neurons with that of the ChAT neurons. In the rhesus monkey (Macaca mulatta), the NBM, virtually all of the CaBP neurons contain ChAT, whereas about 70 to 80% of the ChAT neurons contain CaBP. On the other hand, CaBP does not co-localize with ChAT in the rat (Sprague-Dawley) NBM. The rat CaBP neurons are fewer in number and smaller in size than the ChAT neurons. The differences in the expression of CaBP immunoreactivity in the rat and monkey NBM suggests that vitamin D-dependent calcium homeostasis may have different roles in the primates and the rodent basal forebrain functions. (Supported by grants from USPHS AG005944, BHSR RR05423, the Alzheimer's Disease and Related Disorders Association, and the Neuroscience Center of the University of Tennessee, Memphis.)
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

THURSDAY PM

BASAL GANGLIA AND THALAMUS VII

105.6

CALBINDIN-D28K AND PARVALBUMIN IN PRIMATE BASAL GANGLIA.

The distribution of cell bodies expressing either calbindin-D28K (CB) or parvalbumin (PV) immunoreactivity (IR) in basal ganglia of squirrel monkeys (Saimiri sciureus) was studied on contiguous sections incubated with monoclonal antibodies to CB and PV (M.R. Collin). In the striatum, medium-sized CB-IR cells occurred in very large number and appeared strictly confined to the extrastriatocortex, as identified by its intense acetylcholinesterase staining on adjacent sections. Less numerous medium-sized PV-IR neurons were also distributed in a patch-like manner in the striatum, but the correspondence with the striatal organization was less striking. CB-IR neurons in the dorsal portion of the striatum were less intensely stained than those in the ventral portion, whereas the inverse occurred for neurons expressing a moderate level of the pallidal level of PV. Both segments were devoid of CB-IR but displayed very strong PV-IR. The neurons of the subthalamic nucleus were also markedly enriched with PV but displayed only very light CB staining. In the substantia nigra (SN)-ventral tegmental area (VTA) complex, CB-IR cells abounded in the VTA and in the dorsal tier of the pars compacta of SN, but were absent in the ventral tier of the pars compacta and in the entire pars reticulata of SN. In contrast, numerous PV-IR neurons occurred in pars reticulata and pars lateralis of SN, but none were found in the pars compacta and VTA. These findings reveal that PV and CB distribution in primate basal ganglia are strikingly complementary, suggesting a synergetic role for these two calcium binding proteins in basal ganglia function. [Supported by grants from MRC and FRQS].

105.7

RECIPROCAL CONNECTION BETWEEN THE TWO PALLIDAL SEGMENTS IN PRIMATES.

Studies with the lectin Phaseolus vulgaris-leucoagglutinin (PHA-L) revealed the existence of reciprocal connections between the external (Gpe) and internal (Gpi) segments of the pallidum in the squirrel monkey (Saimiri sciureus). Small intravenous injections of PHA-L in the dorsomedial half of Gpe resulted in widespread labeling of Gpe and Gpi. Both smooth and labeled fibers arose from the injection site, traversed the internal medullary lamina and invaded the dorsolateral half of Gpe. The coarse fibers passed through Gpi to terminate within the subthalamic nucleus and the substantia nigra. In contrast, the finer terminal branching of these fibbers was more pronounced within Gpi. They were more densely and arborized in a pericellar basket-like pattern around the somata and proximal dendrites of Gpi neurons. Typically, one perikaryon was innervated by a single axon displaying numerous rather large varicosities reminiscent of terminal boutons. Conversely, PHA-L injection in the central core of Gpi led to significant retrograde fiber labeling in the dorsomedial half of Gpe. These fibers displayed long and sparsely distributed segments proximally, and branched rather frequently distally making contact en passant with several Gpe cell bodies. Current electron microscopic studies should reveal more about the type of contact established by this short pallidal-pallidal interconnection system, which could play a crucial role in the functional organization of the basal ganglia. [Supported by grants from the MRC and FRQS].

105.9

THE EXISTENCE OF A MARGINAL DIVISION IN THE MONKEY STRIATUM. S. Y. Shu and X. Y. Bao. Dept. of Neurobiology, Inst. of Neuroscience, Xi'an, China.

In the rat, a band of densely packed medium sized fusiform cells has been found at the caudal border of the striatum and named marginal division (Adell and Swanson, 1988). The marginal division has special morphology, immunohistochemistry and projection patterns, distinguishing it from the rest of the striatum. The present study is to investigate whether a marginal division is in the monkey striatum. A part of the putamen (Macaca mulatta) brain including the putamen and globus pallidus was sectioned and the sections were processed immunohistochemistry of L- enkephalin (L-ENK), neurotensin (NT), and cholecystokinin (CCK). L-ENK, CCK and NT immunoreactive fibers and terminals were more densely distributed in the caudo-medial part of the putamen than in the rest of the putamen. The neuronal somata are mostly fusiform in this region and there are L-ENK-IR terminal plexuses. Based on the morphology, immunohistochemistry, and position of this region, it is very possible that a special marginal division, similar to the marginal division of the rat, also exists in the caudo-medial border of the putamen adjacent to the globus pallidus in monkeys.
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

The supplementary eye field (SEF) and the frontal eye field (FEF) are two regions distinct but interconnected areas of the frontal lobe involved in the control of saccadic eye movements. Microinjection of FEF elicits vector-coded saccades, whereas microinjection of SEF elicits movements whose amplitude and direction are eye-position dependent. Both of these cortical eye fields send projections to the striatum, another region known to process visual and oculomotor information. In the experiments described here, we asked whether the SEF and FEF convey their oculomotor representations to the same or to different striatal sites.

We compared the corticostriatal projections of the two eye fields in 5 cynomolgous monkeys. Guided by responses to cortical microinjection, we placed either of the two anterograde tracers, [3H]methylene or horseradish peroxidase - wheat germ agglutinin (HRP-WGA), into each field. Serial section analysis of the striatum demonstrated a patchy projection to the striatal matrix from each of these areas that is restricted primarily to the body of the caudate nucleus and the cell bridges between the caudate nucleus and putamen. The projections were often intermingled, but they were largely non-overlapping.

The striatum exerts critical effects on the nigrostriatal control system that modulates activity in the superior colliculus. Our results indicate that there may be separate corticostriatal circuits subserving this control in the basal ganglia. Supported by NIH grant EY02866.


[14C] deoxyglucose autoradiography was used with electrical stimulation of the cortex to map corticostriatal fields of activation. Awake animals (n=23) were stimulated in forelimb MI cortex, vibrissa SI and hindlimb sensorimotor cortex. Cortical and striatal activation extended several mm anterior-posterior. Image analysis was used to define and localize activated striatal regions. Segregation of forelimb, vibrissa and hindlimb activated regions was significant in striatum. However, vibrissae sensory and motor cortex stimulation resulted in extensive overlap of activation in ipsilateral striatum, suggesting extensive sensory-motor integration. Of potential great interest is that activation contralateral to the stimulation side was .4 mm ventral to the ipsilateral activation, suggesting a place code for ipsilateral and contralateral (left/right) information in the striatum. The results confirm a definite somatotopy in striatum, but with widespread fields, especially vibrissae.

DISTRIBUTION OF BASIC FIBROBLAST GROWTH FACTOR IN THE KINE LATE STRIATUM. Rosalinda C. Roberts, Cornelio G. Cady, Seth F. Finklestein and Marian DiFiglia. Massachusetts General Hospital, Boston MA. 02114

In neocortical cultures, basic fibroblast growth factor (bFGF) enhances neuron survival and protects striatal neurons from glutamate toxicity (Prease et al., this meeting). Although bFGF has been localized to the number of OX6 areas in adult brain little is known about its distribution in the neostriatum. In the present study, bFGF immunoreactivity was localized in the adult rat striatum. bFGF positive neurons were abundant throughout the striatum, with only small pockets of tissue lacking labeled cells. Many medium and large neurons were labeled, however not all cells in each subcategory were positive. At the EM level immunoreactivity, visualized with both DAB and colloidal gold, was deposited in patches throughout the cytoplasm of somata and dendrites. Reaction product was most often associated with neurofilaments and microtubules. Light or no label was found in glia or axon terminals, however bFGF positive axon initial segments were occasionally seen. Thus, bFGF may be an intrasomatic growth factor that concentrates in the mature striatum; its subcellular distribution suggests that it is not actively secreted from striatal neurons. NIH grant NS06367 to MG.


To quantify the relative contributions of projections from somatosensory cortical areas to the striatum, we have calculated how relative corticostratal magnification factor varies with cortical area and body part representation. In 15 squirrel monkeys we made 25 injections of anterograde tracers ([3H]methylene or HRP-WGA) into the mouth (n=8), hand (n=10), and foot (n=7) representations of area 3a (n=6), area 3b (n=13), and area 1 (n=6). Injection sites were guided by multisite recording of somatosensory receptive fields. Cross-sectional areas of labeled tissue were measured on a computer image analyzer from 40 um sections taken 480 um apart. We defined the corticostriatal magnification factor (M) for a cortical site as the ratio of the volume of labeled striatum to the volume of the injection site in cortical layers III through V.

Three-way analysis of variance showed that neither the body part representation (M) nor the injection site (n) was significant. However, the interaction between the two, M x n, was significant (p<0.005). The projection from area 3a to the striatum (M=6.3±0.93) was about twice as broad as those from area 3b (M=3.1±0.34) and area 1 (M=2.1±0.27). (All values are means ± SEM.)

This result is of interest because area 3a represents primarily deep (muscle spindle) receptors, and may be more important for position sense than are areas 3b and 1, which represent primarily cutaneous sensation. The greater corticostriatal magnification of area 3a thus supports the view that the striatum is more concerned with posture and movement control than with fine touch. Supported by Javits Award 5 RO1 25259-02 and the Seaver Institute.

DESTRUCTION OF BASIC FIBROBLAST GROWTH FACTOR IN THE KINE LATE STRIATUM. Rosalinda C. Roberts, Cornelio G. Cady, Seth F. Finklestein and Marian DiFiglia. Massachusetts General Hospital, Boston MA. 02114

In neocortical cultures, basic fibroblast growth factor (bFGF) enhances neuron survival and protects striatal neurons from glutamate toxicity (Prease et al., this meeting). Although bFGF has been localized to the number of OX6 areas in adult brain little is known about its distribution in the neostriatum. In the present study, bFGF immunoreactivity was localized in the adult rat striatum. bFGF positive neurons were abundant throughout the striatum, with only small pockets of tissue lacking labeled cells. Many medium and large neurons were labeled, however not all cells in each subcategory were positive. At the EM level immunoreactivity, visualized with both DAB and colloidal gold, was deposited in patches throughout the cytoplasm of somata and dendrites. Reaction product was most often associated with neurofilaments and microtubules. Light or no label was found in glia or axon terminals, however bFGF positive axon initial segments were occasionally seen. Thus, bFGF may be an intrasomatic growth factor that concentrates in the mature striatum; its subcellular distribution suggests that it is not actively secreted from striatal neurons. NIH grant NS06367 to MG.


The cortexebral cortex projects massively to the dorsal-lateral part of the caudate putamen (striatum), a structure primarily involved in the control of movement and cognition. A subset of cortico-striatal excitatory inputs has been shown to terminate upon a category of medium-sized aspynerve internurons expressing both neuropeptide Y (NPY) and somatostatin (SOM). Previous immunohistochemical studies have shown that the number of neurons stained for NPY and SOM increase after unilateral cortical lesion in the rat (Kerkerian et al, Eur. J. Neurosci. 2, 1990; Salin et al Brain Res. in press). Our goal was to determine whether unilateral NPY and SOM gene expression was affected after cortical deafferentation. Fronto-parietal cortex was lesioned in rat by superficial thermoablation. Lesioned and control rats were sacrificed 5 or 21 days after lesion. The brains were cut on a cryostat, (10um-thick coronal sections) and processed for in situ hybridization histochemistry using a 35S radiolabeled cRNA probe. The number of labeled cells per striatal area was enumerated for each brain by lamination per individual neuron (summary of pixels occupied by silver grains) were measured with an image analysis system (Morphon). In lesioned rats, the number of cells containing NPY and SOM mRNA relative to the intensity of labeling per single neuron (summarized in histograms and graphs) were measured with an image analysis system (Morphon). The relative increase of the mRNA and peptide levels suggest that the lesion of the cerebral cortex by thermocoagulation results in activation of NPY/SOM striatal interneurons. Supported by BNS 85-16841.

Previous studies (Uhl et al. J. Neurosci. 88) have shown a decrease in striatal enkephalin (Enk) mRNA levels after cortical lesions in rats. We have further investigated the effect of lesions on the levels of mRNA encoding glutamic acid decarboxylase (GAD), Enk and substance P (SP), and the corresponding peptides in the basal ganglia. Frontoparietal cortical lesions bilaterally or in the thalamus, and the animals were sacrificed 5, 21 and 90 days after the lesion. Cryostat sections (10um) were processed either for immunohistochemistry with 125I labeled antibodies or for in situ hybridization with 35S radiolabeled cRNA probes. Sections were exposed to X ray film and the optical density of the autoradiographic signal measured. No striatal atrophy was observed at any time after the lesion. SP and GAD immunoreactivity was reduced in the internal (entopeduncular nucleus) and external (globus pallidus) pallidum respectively. This correlates with data on human cortical lesions or injuries (Bates et al. Neurosci. Abs. 15 861 89). Enk and GAD mRNA levels increased in the striatum at all survival times but SP mRNA increased only at 5 days post-lesion. The results suggest a paradoxical increase in the synthesis of striatal efferent neurotransmitters after disruption of excitatory cortical pathways. The discrepancy observed between our results (by thermocoagulation lesion) and data of Uhl et al. (by aspiration lesions) could be explained by different experimental or compensatory post-lesional processes. The prolonged and marked effects observed on Enk containing-neurons suggest that they may be a preferential target of cortico-striatal inputs. Supp by BNS 86-16841.


The role of the dopaminergic nigro-striatal pathway in the regulation of GAD and SOM mRNA in neurons of the pallidum was investigated by in situ hybridization histochemistry (ISHH). Adult rats received an unilateral injection of 6-OHDA (6 mg in 2 μl) in the substantia nigra. After 7 days, sections were prepared for ISHH with a 32P labeled cRNA for GAD (M. M. Scheller, Dept. Psychiatry, MA) or SOM (T. H. Goldman). In the globus pallidus of 6-OHDA-treated rats, the number of labeled cells was higher (29 ± 2 cells per mm2) on the side ipsilateral to the lesion and lower contralateral (10 ± 2.8) and for controls (20 ± 2.8). The intensity of labeling per cell was also higher in the globus pallidus ipsilateral to the lesion. In the entopeduncular nucleus of 6-OHDA-treated rats, the number of GAD-labeled cells was higher on the side ipsilateral to the lesion (17 ± 2.2) whereas the contralateral side had a number of labeled cells similar to controls (9 ± 1.5). By contrast, there was a massive increase (3.4 times) in the number of SOM-labeled cells, and intensity of labeling per cell, on the entopeduncular nucleus ipsilateral to the lesion (as compared to contralateral or control). These results suggest that removal of the dopaminergic neurons of the substantia nigra increases the expression of GAD mRNA in both pallidal segments in the rat and affects dramatically the expression of SOM mRNA in the entopeduncular nucleus. Supp by BNS 86-16841 and MIH44894 and the PMAR (JJS).

506.6 REGULATION OF NEUROPEPTIDE EXPRESSION IN THE NUCLEUS ACCUMBENS OF THE RAT. P. Voorn, C.R. Geffen, Lab of Cell Biology, NIMH, Bethesda, MD 20892. Dept. Anatomy, Vrije University, Amsterdam, the Netherlands.

Enkephalin (ENK) and substance P (SP) immunoreactivity (IR) patterns in the nucleus accumbens (NA) are heterogeneous, showing areas of heavy, moderate, or light immunostaining. The distribution of the enkephalinergic and the GABAergic neurons (GAD) in situ hybridization histochemistry (ISHH) of ENK- and SP-mRNA with oligodeoxynucleotides showed that the heterogeneous IR-patterns in the shell region, but not in the core region of the NA can be recognized also in the distribution of ENK- and SP-mRNA. In the caudate-putamen decreased dopaminergic neurotransmission or lesioning of the nigro-striatal pathway led to dramatic alterations in peptide- and mRNA levels for ENK and SP. The present study focuses on possible regulation of ENK- and SP synthesis in the NA by dopaminergic and/or alcohoric action and in the hippocampus. Quantitative ISHH and IHC were used to evaluate changes in the levels of ENK- and SP-mRNA after unilateral lesions of the dopaminergic fibers in the ventral tegmental area (VTA) and the nigro-striatal transaction of the fornix. Results show that 10-14 days after 6-OHDA lesion the level of expression of ENK is increased and that of SP is decreased on the lesioned side compared to respective levels on the non lesioned side. Dopaminergic regulation may act through direct inputs to the ENK- or SP-containing cells, but may also be relayed through cholinergic interneurons. A dopamine-achetholine interaction was studied at the morphological level by employing ISHH for the D2 receptor (Bunaw et al. Nature 336, 1988) and IHC for choline-achetholine reatherase. Results show that cholinergic neurons in the caudate-putamen express the D2 receptor, whereas in the NA not all cholinergic neurons appear to express the D2 receptor.


Cocaine and amphetamine are psychomotor stimulant drugs that produce dramatic long-term changes in behavior. Many of these effects are thought to be mediated by dopaminergic mesostriatal systems. Here we tested the possibility that these drugs activate immediate early genes (IEGs) in the striatum. Adult rats were treated with intraperitoneal amphetamine (5 mg/kg), cocaine (25 mg/kg), or saline, and were perfused after 45 min. 6 hr. Sections through the striatum were stained for fos-like immunoreactivity and for c-firm-like immunoreactivity and for c-firm-like immunoreactivity and for c-firm-like immunoreactivity. Amphetamine and cocaine, but not saline, induced highly specific patterns of c-firm expression in dorsal and ventral striatum. Immunoreactivity appeared in nuclei of medium-sized and large neurons by 1 min after drug and declined by 6 hr. Cocaine induced c-firm both in striosomes and in matrix. By contrast, amphetamine induced a striking strisome-predominant pattern in the rostral caudoputamme and the dorsolateral substantia nigra. In the latter, synthesis with oligodeoxynucleotide probes established that the induction involved c-firm transcription. Induction of c-firm-like immunoreactivity by each drug was blocked by pretreatment with the D1 dopamine antagonist SCH 23390 (0.01 mg/kg), but only cocaine-elicted induction was blocked by reserpine (10 mg/kg).

We conclude that some of the physiological and behavioral effects of psychomotor stimulant drugs may be mediated by c-firm transcription in striosomes and matrix. Supported by The Seaver Institute, NIH NS 25529, The United Parkinson Foundation, NARSAD and MRC of Canada.

506.8 ACTIVATION OF RAT STRIATAL c-FOS BY DIRECT INFUSION OF DOPAMINERGIC AGONISTS AND FOSRELIN. H.A. Robertsonthet, A.M. Saydil, R. Moretallia, D. Robertson, and M.E. Feinmannel, Dept. Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada B3K 687 and Dept. Brain & Cog. Sci., MIT, Cambridge, MA 02139, U.S.A.

Recently we demonstrated that systemic injections of D-selective dopamine agonists activate the immediate-early gene c-fos in the caudate-putamen and nucleus accumbens ipsilaterally to the injection (Robertson et al. Brain Res. 503: 346, 1989). To characterize further this response, we have now investigated the effects of direct infusion of agonists into the striatum in naive rats, in rats with unilateral 6-OHDA lesions and in rats pretreated with the dopaminergic depleting drug, reserpine. Drugs were slowly infused under pentobarbital anesthesia, and two hours later, rats were perfused for immunohistochemical localization of c-fos protein. Infusions of SKF-38393 (1 μg/5 μl) or LY-171555 (0.05 μg/5 μl) produced marked c-fos activation in 6-OHDA and reserpine-lesioned rats but had no effect in naive rats. This response was attenuated by the D1 dopamine antagonist SGN-503 (0.5 mg/kg, i.p.). Forskolin produced c-fos activation in both naive and treated animals, and this was unaffected by SGN-23390. In all animals, c-fos immunoreactivity was confined to the striatum.

In agreement with our earlier studies, the D2-selective agonist LY-171555 did not induce c-fos activation when infused bilaterally into the striatum. These results suggest that the effect of the D2 agonists is direct and may be mediated by dopamine-sensitive adenylyl cyclase.

(Supported by MRC of Canada and Javits NS 25329.)
506.0
THE EFFECTS OF ACUTE ANTIPSYCHOTIC DRUG TREATMENT ON MONOAMINE SYSTEMS IN THE NUCLEUS ACCUMBENS CORE AND SHELL. D. A. Cameron and A. Y. Depch, Department of Pharmacology and Toxicology, Stony Brook University, New York, NY 11794.

Recent anatomical studies indicate that the nucleus accumbens (NAC) can be divided into the core and shell (C&S) regions. The NAC has cholinergic (Ch) afferents distributed to the NAC and C&S. These afferents converge on the Ch terminals, which are located in the shell and core of the NAC. The Ch terminals are distributed to the NAC and C&S in different patterns and densities. The NAC is innervated by the pedunculopontine tegmental nucleus (PPT) and the laterodorsal tegmental nucleus (LDT), which are cholinergic nuclei. The NAC is also innervated by the substantia nigra pars reticulata (SNr) and the substantia nigra pars compacta (SNc), which are dopaminergic nuclei. The NAC is innervated by the subthalamic nucleus (STN) and the ventral tegmental area (VTA), which are glutamatergic nuclei.

506.11

We sought to identify which neostriatal cells are responsive to niada receptor activation. The niada agonist quinpirole (quin) (100 nCi) was injected into the rat striatum (n=10). 2h later immunohistochemistry was performed using affinity-purified antibodies against the 127-154 fragment of Fos, a c-fos reporter of gene transcription. Results: QA injection increased nuclear labeling of Fos throughout the neostriatum: CSF infused in the striatum lateralized the injection site of Fos. injection of QA with the niada receptor antagonist APV induced Fos expression. Fos was localized predominately to medium-sized neurons, including cells reactive for NADPH diaphorase; most large neurons were devoid of nuclear labeling. Ultrastructural examination verified that Fos was confined to the nuclei of medium-sized neurons with undifferentiated and indented nuclei; nuclear membrane was distended unevenly in the karyoplasm and notably in patches along the inner face of the nuclear membrane. Implications: Medium-sized spiny and aspiny neurons have a high density of niada receptors and activation of niada receptors in these neurons contributes to the regulation of o-c-fos gene expression.

Supported by the NSF and NIH.

506.12
Muscimurine Receptors Modulate Apomorphine Induction of Dynorphin in Striatal Patch Neurons. J.B. Dumas and J.F. McGinty, Department of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville N.C.

Previous studies have reported that injection of the dopamine (DA) agonist, apomorphine (APO), increases dopamine immunoreactivity (DAIR), and mRNA for non-NMDA receptors (nNMDA) in striatal patches (Li et al., J. Neurosci., 1994). The present study was designed to investigate whether cholinerigic tone affects the APO-induced DAIR in striatal patches. The study was conducted to investigate whether cholinergic tone affects the APO-induced DAIR in striatal patches. The study was conducted to investigate whether cholinergic tone affects the APO-induced DAIR in striatal patches. The study was conducted to investigate whether cholinergic tone affects the APO-induced DAIR in striatal patches. The study was conducted to investigate whether cholinergic tone affects the APO-induced DAIR in striatal patches.

The results demonstrate that nicotinic agonists do not significantly affect APO-induced DAIR in striatal patches. The results demonstrate that nicotinic agonists do not significantly affect APO-induced DAIR in striatal patches. The results demonstrate that nicotinic agonists do not significantly affect APO-induced DAIR in striatal patches. The results demonstrate that nicotinic agonists do not significantly affect APO-induced DAIR in striatal patches.

Supported by DB 081300, NS19613.

507.1

We have recently described several categories of mesopontine cholinergic neurons related to the genesis and brainstem-thalamic transfer of pontine-geniculo-occipital (PGO) waves (Steriade et al., 1990). Neurones, in press). As one of these cellular types discharge during sleep and spontaneous episode bursts over a background of decreased firing rate during REM sleep, we hypothesized that these PGO-on bursts are generated by low-threshold spikes desynchronized by membrane hyperpolarization and further suggested that GABAergic SNR cells represent one of the possible sources of hyperpolarization in peribulbar (PB) neurons.

To test this hypothesis, we recorded single SNR cells in chronically implanted, naturally sleeping cats. SNR cells were antidromically identified from the PB area and ventromedial thalamic nucleus. Quantitative data were obtained from a sample of 18 SNR cells recorded during waking (W), EEC-synchronized sleep (S) and REM sleep. Spontaneous discharges were similar in W (26.3 Hz) and S (28.8 Hz), but increased significantly during REM sleep (42.8 Hz). In all states, SNR neurons discharged tonically, with an absence of high-frequency bursts and a symmetrical interburst interspike histogram. Only 2 of these SNR cells increased their tonic firing 70-100 ms prior to the PGO-like wave. These data suggest that a population of SNR cells may exert tonic and/or phasic inhibitory actions upon a class of PB neurons that fire PGO-on bursts crowning the low-threshold spike. Supported by MRC grant MT-3869.

507.2

The effect of 5-HT on neurons of the pedunculopontine (PPT) and laterodorsal tegmental (LDT) nucleus was studied with intracellular recording methods in brain slice preparations from guinea pig and rat. Cholinerigic neurons were identified physiologically and morphologically by combined intracellular injection of Lucifer yellow or biocytin and histochimical staining for NADPH diaphorase (Leonard and Llinas, '88, Soc. Neurosci. Abstr. 14: 249), which selectively labels mesopontine cholinergic neurons (Vincent et al., '83, Neurosci. Letts. 43: 31-36), and focal application of 5-HT on physiologically identified cholinerigic neurons (type II cells, Leonard and Llinas, '89, In: Brain Cholinergic Systems, Steriade and Bieseld, Eds.) produced a transient suppression of firing and membrane hyperpolarization. Bath application of 5-HT produced a maintained hyperpolarization whose magnitude varied with 5-HT concentration (3-30uM). The hyperpolarization was unaffected by Ringer containing TTX and low Ca2+ 2mM Co3+, indicating a direct action of 5-HT on mesopontine neurons. The hyperpolarization reversed near the potassium equilibrium potential and was accompanied by a large decrease in membrane resistance suggesting the activation of a potassium current. These results imply that mesopontine cholinerigic neurons are directly inhibited by their serotonergic afferents and suggest that they become disinhibited during REM sleep when serotoninergic neurons exhibit greatly reduced discharge rates. Supported by NIMH/N51742 and a grant from the American Parkinson Disease Association.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
507.3 LATERAL HYPOTHALAMIC PROJECTIONS TO THE PEDUNCULOPONTINE TERMINAL NUCLEUS AND ADJACENT MIDBRAIN TERMINGMENT IN THE RAT. T.L. Steinmetz, R. McEwen, and J.L. Price, Dept. of Psychol., Univ. of Chicago, Chicago, IL 60637.

The cholinerigic neurons of the pedunculopontine terminal nucleus (PPT) are thought to modulate behavioral arousal through its widespread projections to the thalamus and brainstem nuclei. Recent retrograde tracing studies suggest that this nucleus receives information from a variety of sources, including the posterior lateral hypothalamic area (LHAp). This study was designed to investigate the connections of this nucleus. PPT neurons were retrogradely labeled by injecting a horseradish peroxidase substrate into the PPT. The injections were made in mediodorsal and centromedial nuclei. Of the labeled neurons, 90% were in the midbrain, mainly in the periaqueductal gray, with others scattered throughout the lateral hypothalamus and ventral tegmental area. The labeled neurons were found to project to areas of the thalamus, basal forebrain, and brainstem nuclei. This study suggests that the PPT is a major source of cholinergic input to the thalamus, basal forebrain, and brainstem nuclei.


Presynaptic terminals from the brainstem to MD were studied with the electron microscope in the rat. Silver tartrate, modified transport of WGA-HRP. Labeled axons were seen mainly in the lateral part of MD, with few axons visible in the central part. The boutons arising from SC were smaller (<1 μm in diameter), formed symmetrical synaptic contacts with dendrites, and contained round synaptic vesicles. The axon terminals of SC resembled those of other brainstem nuclei, with terminal boutons 2-4 μm in diameter, a high degree of collateralization, and occasional symmetrical contacts.

507.5 LDT-PPT CHOLINERGIC NEURONS COLLABORATE TO TWO THALAMIC TARGETS. D. Shimony, J. Velazquez-Moretuna, and C. Floyd,* San Diego VAMC and UCSD, La Jolla, CA 92039.

Cholinergic neurons in the lateral dorsal tegmental (LDT) and pedunculopontine tegmental (PPT) nuclei have been shown to heavily innervate the thalamus. In order to determine whether single LDT-PPT cholinerigic neurons simultaneously project to two thalamic targets, in vivo anterograde and retrograde autoradiographic injections of 2,3,5-triphenyltetrazolium chloride (TTC) and horseradish peroxidase (HRP) were made into the thalamic lateral geniculate nucleus (LGN), lateral geniculate nucleus (LGN), and the upper lateral geniculate nucleus (ULG). The injections were made in the thalamus, basal forebrain, and brainstem nuclei. The labeled neurons were found to project to areas of the thalamus, basal forebrain, and brainstem nuclei. This study suggests that the PPT is a major source of cholinergic input to the thalamus, basal forebrain, and brainstem nuclei.


Interconnections between raphe nuclei have long been implicated from retrograde axon tracing studies. In the present study, injections of the lectin PHA-L in various raphe nuclei and mesencephalic targets produce networks of anterogradely labeled varicosities throughout the brainstem and mesencephalic nuclei. The PHA-L labeling pattern is similar to that observed in previous studies. However, the present study demonstrates that the PHA-L labeling pattern is more extensive and dense than that observed in previous studies. This suggests that the PHA-L labeling pattern is more extensive than that observed in previous studies.
SOT.9
BRAINSTEM PROJECTIONS FROM THE NUCLEUS OF THE SOLITARY TRACT IN THE RAT. E.T. Cunningham, Jr., and P.E. Sawchenko. The Salk Institute for Biological Studies, La Jolla, CA 92037.

A retrograde transport and immunohistochemical technique were used to characterize the organization of brainstem projections from the nucleus of the solitary tract (NTS) in the rat. The results show that:

1. NTS neurons in the rostral tegmental region give rise to an extensive set of intrinsic projections that almost invariably involve "associational" and "commisural" regions within that subregion on the ipsilateral and contralateral sides.

2. The central tegmental nucleus (NTS) projects to a strong input to the ventral and lateral parts of the nucleus. The central subnucleus, in contrast to other parts of the NTS, gives rise to, and receives, only sparse intrinsic inputs.

3. The central subnucleus of the NTS projects to the intermediolateral nucleus of the spinal cord, the caudal ventral lateral part of the hypoglossal nucleus, and to the region of the n. ambiguus.

4. The central subnucleus gives rise to a dense, primarily ipsilateral, projection to the rostral, part of the central nucleus, the pontine nucleus.

5. Of most of the caudal, medial half of the NTS, including the commissural portion, project heavily to the dorsal motor nucleus of the vagus nerve (DMX).

6. The central gray (CC) receives projections primarily from those parts of the caudal and commissural NTS known to contain a2 and c2 catecholamine cell groups. The a1 and c1 catecholamine cell groups in the ventrolateral medulla receive their heaviest and most direct input from the commissural part of the NTS.

7. The parabrachial nucleus receives a dense input from virtually all parts of the NTS, with the exception of the central subnucleus. Together, these results provide evidence for topographically discrete pathways from the NTS to a number of motor and relay nuclei important in autonomic, neuroendocrine and orofacial function.

SOT.10
IN VITRO RAT BRAINSTEM SLICES REVEAL GUSTATORY NEURON TYPES. R.M. Bradley and R.D. Sweeney. School of Dentistry, Michigan State University, East Lansing, MI 48824.

Using intracellular recordings and bicynicin injections, we are defining the intrinsic membrane properties of neurons in the gustatory zone of the solitary tract nucleus (NTS). Three distinct neuron types were separated using current injection pulse paradigms in rat brainstem in vitro slice preparations. Injections of depolarizing current produced a low frequency, regular pattern of responses. A: a high frequency, regular response in Type II neurons; and a burst-like pattern of response in Type III neurons. Furthermore, in both Type I and III neurons, depolarizing current produced a delay in the initiation of the first spike. This delay was dependent on both the magnitude and duration of the depolarizing prepulse. Preliminary reconstructions of biocytin-filled Type III neurons indicate that these physiologically defined neuron groups also have distinctive anatomical characteristics. The presence of neuron types in the gustatory NTS suggests that these groups have different roles in processing gustatory information.

Supported by N.I.H. Grant DC0288.

SOT.11

We report on the effects of hypercapnia (CO2 76-656, 1987; Neurosci., in press) on neurons in the nucleus of the solitary tract (NTS) and dorsal motor nucleus of the vagus (DMX) in the rat. Using intracellular microelectrode recordings and biocytin injections, we are determining the direct effects of hypercapnia on neurons of the dorsal vagal complex (medullary respiratory, vagal and splanchnic nuclei). The results indicate that distinct, clearly defined neuronal populations are activated by hypercapnia. In the ventrolateral medulla, CO2-sensitive neurons were observed to increase firing rate and to increase afterhyperpolarization following hypercapnic stimulation. In the dorsal motor nucleus, CO2-sensitive neurons were observed to decrease firing rate and to increase afterhyperpolarization following hypercapnic stimulation. These results provide evidence for the existence of CO2-sensitive neurons in the medullary respiratory and splanchnic nuclei of the rat.

SOT.12
IN VITRO STUDIES OF HYPOXIC MUSCLE INTEGRATION OF MEMBRANE PROPERTIES IN THE DORSAL VAGAL COMPLEX. R.A. Gallman, J.B. Dean, and D.E. Millhorn. UNC, Chapel Hill, NC 27599.

The present study was undertaken to determine the direct effects of hypoxia on neurons of the dorsal vagal complex (medullary respiratory, vagal, and splanchnic nuclei). The results indicate that distinct, clearly defined neuronal populations are activated by hypoxia. In the ventrolateral medulla, hypoxia-sensitive neurons were observed to increase firing rate and to increase afterhyperpolarization following hypoxic stimulation. In the dorsal motor nucleus, hypoxia-sensitive neurons were observed to decrease firing rate and to increase afterhyperpolarization following hypoxic stimulation. These results provide evidence for the existence of hypoxia-sensitive neurons in the medullary respiratory and splanchnic nuclei of the rat. Together, these results suggest that the medullary respiratory and splanchnic nuclei are important in the neural control of respiration and circulation.

SOT.13

The connectivity of the ferret dorsal vagal complex was studied using retrograde or WGA-HRP delivery to microprobes. Animals were perfused transcardially with buffered saline followed by 2.5% glutaraldehyde in 0.1M phosphate buffer after 24-48 hours. Tissues were cut at 50um on a freezing microscope and reacted with tetramethyl benzidine. In different animals, injections were restricted to the AP immediately adjacent to the AP centered in the solitary nuclear complex, or involved the AP together with the adjacent tegmentum. In cases with injections limited to the AP, labeled fibers could be traced only into the adjacent solitary complex, specifically the medial and subnucleus medialis. Those nuclei also contained labeled peri-karya. After injections centered in the solitary complex, labeled peri-karya occurred in the AP, parabrachial nucleus, nucleus ambiguus, nucleus of the hypoglossal and in the substantia innominata. Efferently labeled fibers were followed to the paraventricular nucleus. We conclude that in ferrets, ascending brainstem pathways from the AP relay in the solitary complex.

SOT.14

The ferret is becoming increasingly important in neurophysiological studies. Since we are using this animal for studies of G.I. tract function, it was necessary to examine the distribution and localization of various neuropeptides and 5-HT in the ferret brainstem. In this study, the localization of the 5-HT and hypothalamic (III) nuclei were confirmed using retrograde tracings with HRP applied to the trigeminal or vagus nerve. The central motor and sensory divisions were confirmed by injections of antibodies against substance P (SP), calcitonin gene-related peptide (CGRP), enkephalin (ENK) and serotonin (5-HT), and processed using the PAP procedure. All immunoreactivity (IR) appeared in fibers and/or terminals. In the nTS, SP, and to a lesser extent, CCRP-IR were present in discrete areas, suggesting localization within subnuclei. Dense SP- and CCRP-IR were also found in the DMV. In contrast, serotoninergic IR was evenly distributed, whereas 5-HT-IR was sparse and scattered, but both were found throughout the DVC. The only IR cells bodies seen were those containing 5-HT and SP, and those containing the brainstem (approx. 2.0 mm rostral to obex) contained primarily CCRP-IR, to a lesser extent 5-HT-IR and occasionally SP-IR. Supported by Canadian MRC and Alberta Heritage Foundation for Medical Research.

As a basis for future neurophysiological studies in the ferret, it was necessary to examine the central distribution of various neurochemicals in the trigeminal complex; brain sensory nucleus (MSN) and spinal trigeminal nucleus (pSpV), divided into caudal, interpolaris and oralis. Frozen 40µm serial brainstem sections were cut, incubated with antibodies against substance P (SP), calcitonin gene-related peptide (CGRP), cokethylene (ENK) and serotonin (5-HT), and processed using the PAP procedure. In caudal, dense SP-, CGRP- and ENK-immunoreactive fibers and terminals (IR) were present in lamina I and II, and less densely in lamina V. At ~ 80µm caudal to the obex, the central portion of the SP- and CGRP-IR band in laminae I and II disappeared and an additional band appeared anteriorly to laminae IV and V. In contrast, 5-HT-IR was present over the entire sub-nucleus with the most dense IR in laminae I and II. In caudal interpolaris, SP-, CGRP- and ENK-IR was present to the dorsomedial border and a ventromedial portion of the nucleus. This IR disappeared in rostral interoparals. Sparsely 5-HT-IR was present throughout interpolaris and most dense in a band along the medial border. In oralis, all substances were found in IR fibers crossing the trigeminal tract and as terminals and fibers scattered across the periphery of the nucleus, especially along the dorsomedial border. In MSN, SP-, CGRP- and ENK-IR was localized in the dorsomedial part of the nucleus and 5-HT-IR was evenly distributed throughout. (Supported by Canadian MRC and Alberta Heritage Foundation for Medical Research.)

508.1

OLFACTORY BULB NOREPINEPHRINE MAY BE REQUIRED FOR EARLY OLFACTORY LEARNING.

Weiqian Lin, D.A. Wilson, and R.M. Sullivan. Developmental Psychobiology Laboratory, Dept. Psychology, University of Oklahoma, Norman, OK.

Norepinephrine (NE) is known to be critically involved in a variety of olfactory learning paradigms for acquisition of conditioned neurobehavioural responses. For example, in rats, systemic injections of NE β-receptor antagonists can block olfactory preference conditioning and its neural correlates (Sullivan et al., 1989). The present study limited NE blockade to the olfactory bulb during training, to determine the role of NE in early learning.

On PNS, under cold anaesthesia, pups had a cannula (30 ga) chronically implanted into one bulb and had the contralateral naris occluded. Pups were returned to the litter until PN6, when olfactory conditioning occurred. Pups were trained in one of 3 groups, PAIRED - odor paired with tactile stimulation, RANDOM, or ODOR ONLY. During a 1 min habituation period and the 10 min training session, pups had either saline or 100 µM propranolol infused into the bulb (0.1 µL/min). On PN7, pups were given a behavioral odor preference test or injected with [14C]2-DG and exposed to the conditioned odor. Preliminary results suggest that NE antagonists limited to the bulb block acquisition of a learned behavioral odor preference. Neural correlates of this response are being examined.

508.3

THE EFFECTS OF ALPHA-2 ADRENERGIC DRUGS ON CORTICAL AND HYPOTHALAMIC ELECTRICAL ACTIVITY AND LEARNING/MEMORY IN RATS WITH CORTICAL DYSFUNCTION.


The present set of experiments were undertaken in order to study whether alpha-2 adrenergic agonist (guaifencin) or antagonist (atipamazol) might modulate age-associated cognitive dysfunctions. In study 1, the effects of different doses of drugs on cortical electrical activity (spectral electroencephalogram (EEG) the amount of high voltage spindles (HVS) and hippocampal EEG) was found to be dose-dependent. In study 2, the effects of different doses of drugs on hippocampal EEG were also found to be dose-dependent. Guaifencin (Sandoz Ltd, Switzerland) was injected intraperitoneally (4 µg/kg) and atipamazol (Farmos Ltd, Finland) was injected subcutaneously (0.5 mg/kg). At the treated rats received saline. The most significant findings were:

1) atipamazol (3 mg/kg) increased the number of HVS and improved the performance in the alternation test (testing latency) of aged rats. 2) atipamazol (3 mg/kg) decreased the delta and theta power of rel-lesioned rats.

These results suggest that a selective alpha-2 antagonist may improve cognitive dysfunctions related to aging.

508.2

DPS-4, SOCIAL HOUSING CONDITIONS AND OLFACTORY EXPERIENCE INFLUENCE NOVELTY-INDUCED ANXIETY IN RATS. S.K. Olson, M.R. Deggro, J. Carlisle*, J. Uetz, J.L. Sullivan, S. King and M. Dippel, Department of Psychology, Syracuse University, Syracuse, NY 13244.

Three experiments tested the hypothesis that housing control and DPS-4 treated rats together is stressful and increases anxiety. Rats were injected a., with water or the norepinephrine (NE) neurotoxin DPS-4 on the day of birth, placed at weaning in bedding with either a familiar or novel odor, and observed in three different situations 11-13 days later. Rats housed in the familiar odor in mixed groups (DPS-4 and water) showed abnormally low levels of rearing in the open field, and social interaction with strange rats, indicators of anxiety. These effects were not seen in rats from mixed groups housed in the novel odor. The data suggest that exposure to a novel odor can reduce anxiety. Mixed housing in either odor or novel housed rats' exploratory behavior in the home cage, and also depressed frontal cortex NE levels for DPS-4-treated rats. These data suggest that mixed housing is stressful, particularly for DPS-4 treated rats.

508.4


Peripheral administration of epinephrine modulates memory in a time- and dose-dependent manner. Since epinephrine does not readily cross the blood-brain barrier, it is not likely that it exerts its effects directly in the central nervous system. The present experiments compared the effects of epinephrine and phepinephrine (DPE) on retention of an inhibitory avoidance task and a reversal visual discrimination task in mice. The most effective dose of epinephrine for inducing memory facilitation was 0.1 mg/kg, DPE, a less polar analogue of epinephrine which has a higher tendency to cross membranes, also significantly facilitated memory for both tasks when administered posttraining. The dose-response curve also followed an inverted-U shape with 0.01 mg/kg most effective. The enhancing effects of DPE on memory appear to be mediated by central β-adrenoceptors: the centrally-acting β-noradrenergic antagonist propranolol, but not the peripheral-acting β-adrenoeceptor antagonist sotalol, blocked DPE-induced enhancement of memory. However, sotalol did prevent the effects of epinephrine on memory, suggesting a peripheral site of action for epinephrine. α-Adrenoceptors do not appear to be involved in the facilitatory effects of either DPE or epinephrine on memory; the peripherally-acting α-adrenergic blocker phentolamine did not prevent epinephrine-induced enhancement of memory, while neither phentolamine nor the centrally acting α-adrenergic blockers prazosin or yohimbine blocked the facilitatory effects of DPE on memory. These results support the view that epinephrine initiates its facilitatory effects on memory through the activation of peripheral β-noradrenergic mechanisms, while DPE exerts its effects directly in the brain.
508.5 MEMORY IMPAIRMENTS WITH MEDIAL SEPTAL MORPHINE INJECTIONS: ATTENUATION WITH PERIPHERAL GLUCOSE INJECTIONS. M.E. RAGGOZZI, M.C. PARKER AND P.E. GOLD, Dept. Psychol., U. Virginia, Charlottesville, VA 22903.

β-endorphin injected into the medial septal impairs spatial memory (Bostock et al., Behav. Neurosci., 102, 643). Peripheral glucose injections attenuate these deficits produced by several treatments, including opiate agonists. Here, we determined whether morphine injected into medial septal impairs memory in spontaneous alternation and inhibitory avoidance tasks and whether peripheral glucose administration attenuates the deficits. Rats received morphine sulfate (3 μg in 1 μl) and the medial septal 30 min prior to testing for spontaneous alternation performance. Morphine-treated rats had significantly lower alternation scores than did CSF-injected controls. Glucose (100 mg/kg, IP), administered at the time of morphine injection, blocked the impairment. With treatments as above, rats were subsequently trained in an inhibitory avoidance task and tested for retention 24 hr later. Morphine-treated rats had significantly lower retention scores than did controls and this impairment was reversed by glucose. Thus, morphine injected into medial septal impaired two measures of memory and both deficits were reversed by concomitant peripheral glucose administration. These findings are consistent with the view that circulating glucose levels, directly or indirectly, influence functions in the medial septum. (Supported by ONR N0001489-J-1216 and NIA AG 07648).

508.6 PARALLEL EFFECTS OF THE NMDA ANTAGONIST NPS 12626 ON SLEEP AND MEMORY: REVERSAL OF THE MEMORY DEFICIT WITH NON-NMDA AGONIST. L.S. STONE AND P.E. GOLD. Neuroscience Program & Dept. Psychology, University of Virginia, Charlottesville VA 22903.

We previously demonstrated that NPS 12626 impairs spontaneous alternation (SA) and inhibitory avoidance performance as well as long-term potentiation. In the present study we characterized the sleep patterns. Rats were injected with NPS 12626 30 min prior to a 3-hr recording session. Sleep stages were defined using EEG, EMG, and activity measures. 10 but not 1 mg/kg significantly decreased total sleep (TS), paradoxical sleep (PS) bout duration and the PS/TS ratio. 10 but not 1 mg/kg impaired the SA performance of these animals. The parallel effects of this drug on sleep and memory may reflect a more general impairment of arousal mechanisms important to both.

We previously found that glucose, naloxone, and physostigmine attenuate SA deficits produced by a variety of treatments. In this study we similarly attempted to reverse NPS 12626 - induced deficits. Rats were injected with NPS 12626 (35 mg/kg) 50 min prior to SA testing, and glucose (250 mg/kg), physostigmine (0.01 mg/kg), or naloxone (1 mg/kg) 30 min prior to testing. Each drug reversed the effects of NPS 12626 without having any effect when administered alone. Thus, some effects of NMDA antagonists can be ameliorated by non-NMDA treatments. We currently assessing the effects of these treatments on NPS 12626 - induced sleep deficits. (Supported by AG 07648 and ONR N0001489-J-1216; NPS 12626 generously provided by NOVA Pharmaceuticals).


Glucose enhances performance on memory tasks in elderly humans. To dissociate glucose effects on storage from acquisition, we examined the effects of posttraining glucose on memory in elderly humans. In addition, a preliminary dose-response curve of glucose effects on memory was carried out.

Subjects aged 60-81 (n=23) heard a narrative passage and were given immediate posttraining or pretraining glucose, or saccharin. Recall, measured 36 hours later, was significantly better in both glucose conditions than in the saccharin condition. Next, subjects aged 60-82 (n=10) were given beverages with 10g, 25g or 50g glucose and 50.6 mg saccharin. After ingestion, subjects took memory tests previously enhanced by glucose. The 25g glucose dose significantly improved performance relative to saccharin with a trend for improvement at the 10g dose.

These studies suggest glucose is an enhancement of glucose on human memory. The finding that posttraining glucose enhances memory sensitivity that the treatment acts on memory storage processes. One of memory outcomes that the increase in blood glucose levels, as seen in the 24-hour recall enhancement with both pre- and posttraining glucose. Finally, the selective enhancement at 25g suggests that, as in animal studies, glucose effects on memory are characterized by an inverted-U dose-response curve. (Supported by AG 07648 and ONR N0001489).

508.8 THE MEMORY RETRIEVAL EFFECTS OF IDAZOXAN DEPEND ON THE DEGREE OF FORGETTING. M. Bunsey, J. Horns* and B.J. Strupp. Division of Nutritional Sciences and Department of Psychology, Cornell University, Ithaca, NY 14853.

We previously observed that the effect of AVP-4 on memory retrieval is critically dependent on the accessibility of the memory at the time of injection. A single dose of the peptide enhanced memory at long retention intervals when memory was weak in control rats and impaired memory at short retention intervals, when memory was strongly in controls. This pattern of results suggests that the injected peptide was interacting with endogenous changes corresponding to the strength of the memory. Using the same protocol and memory test (social learning paradigm), the present study was designed to determine if this same pattern of results would be produced by pretest administration of a drug that increases norepinephrine (NE) release, the α2 antagonist idazoxan (IDAZOXAN). This hypothesis was based on the observation that: (1) the mnemonic effects of WP are mediated, in part, by catecholamines, (2) changes in central NE metabolism at retrieval have been shown to correlate with recall, and (3) retrieval has been shown to be facilitated by IDA. Pretest IDA (2 mg/kg) produced the same pattern of results as previously observed with AVP-4-A, suggesting that endogenous NE activity at the time of recall varies with accessibility of the memory.

508.9 THE EFFECTS OF ESSENTIAL ELEMENTS ON LEARNING AND MEMORY IN MICE. Q.S. Deng and Y. Yin.* Dept. of Pharmacology, Nanjing Tieg Dao Medical College, Nanjing, China 210009.

Previous studies showed that essential elements are universally required for development (Frieden, 1974). Biochemists of the essential ultrtrace elements. Plenum Press, 1984). We have examined the hypotheses that learning and memory are closely related to some essential elements. Studies were carried out by injecting mice in a Y-maze. Male mice (25-32 g) were divided into 10 groups (N=15 in each group). Animals were fed with standard food but different water containing various essential elements. The Choice accuracy which was the number of correct choice in 10 trials was adopted. After 3 weeks of testing, Mn, Fe, Zn, Cr and Pb have positive effects on learning and memory in mice. (Supported by Natural Science Foundation of China #389001).

508.10 SCOPOLAMINE INTERACTIONS WITH SELECTIVE D1 AND D2 AGONISTS AND PERFORMANCE IN THE RADIAL-ARM MAZE. E. D. Levin and J. E. Rose. Department of Psychiatry, Duke University Medical Center, Durham, NC 27710.

There is evidence for a reciprocal interaction of dopaminergic (DA) mechanisms with muscarinic systems in terms of cognitive function. The adverse effect of scopolamine in the radial-arm maze (RAM) choice accuracy can be counteracted by DA blockade. This study examined the interactions of D1 and D2 agonists with scopolamine effects on RAM choice accuracy. Eleven adult female Sprague-Dawley strain rats were injected (SQ) 20 minutes before testing with normal saline, scopolamine (0.15 mg/kg), the D1 agonist SKF 38393 (2 and 4 mg/kg), the D2 agonist quinpirole (0.04 mg/kg) and each of the D1 and D2 agonist doses with scopolamine. None of the doses of the D1 and D2 agonists by themselves showed signs of reducing choice accuracy. The D2 but not the D1 agonists slowed the rate of responding. Scopolamine by itself caused both a decline in choice accuracy and an increase in choice latency. These effects were not counteracted by either the D1 or D2 agonists. In fact, the D2 agonists showed a further decline in choice accuracy. The effect of counteracting the effect of scopolamine-induced choice accuracy impairment contrasts with the D2 agonist reversal of the RAM choice accuracy impairment caused by the nicoinic antagonist mecamylamine. (Research supported by a grant from the United Way).
508.11 AMPHETAMINE EFFECTS ON JUMP-UP AVOIDANCE AND CAUDATE Dopamine METABOLISM AS MEASURED BY HPLC IN VIVO DIALYSIS P.H. Janak, S. Shibanoaki*, R. Ishikawa* and M. Martinez, Jr., Dept. of Psychology, Univ. of California, Berkeley CA & Dept. of Pharmacology, School of Medicine, Nihon Univ., Tokyo, JAPAN

Each day for 2 days rats received 1 avoidance trial using a 290µA footshock delivered to the floor of an automated self-jump box 5 min after placement in the chamber. Rats were allowed to escape shock delivery by jumping onto a raised shelf. Immediately after each training trial, animals received AMPH or saline, IP. Retention was tested by training to a criterion 24 hr later. AMPH (1 mg/kg) facilitated acquisition of the jump-up response (F(1,7)=10.53, p<.02). In separate animals implanted with cannulas, caudate dopamine (DA), DOPAC and HVA levels were measured following IP injection of AMPH. Dialysate samples were collected at 20 min intervals for 3 hr after injection and assayed with HPLC-ECD. A 1 mg/kg dose increased striatal DA levels 20-40 min after injection to 518±71.85% of baseline. DOPAC and HVA decreased 40-80 min postinjection by 39±2.67% and 66±3.83%, respectively. Supported by DA06192, DA04795 and DA05375.

508.13 FACILITATION OF LEARNING AND MEMORY FOLLOWING ADMINISTRATION OF THE 5-HT3 RECEPTOR ANTAGONISTS ZACOBRIDE AND DAZOBRIDE IN MICE. H. J. Altman* and R. F. Berntson, Department of Psychiatry, Wayne State University School of Medicine, Detroit, MI 48027, Department of Psychology, Wayne State University, Detroit, MI 48022.

Recent evidence points to the existence of multiple serotonin (5-HT) receptor subtypes within the CNS. Presently, three major subtypes of 5-HT receptors have been identified - designated 5-HT1, 5-HT2 and 5-HT3. Over the past several years this laboratory has been systematically investigating the role(s) each of these purported 5-HT receptor subtypes may play in the mediation of the processes underlying learning and memory. The purpose of the present series of studies was to examine the effects of acute pre-training and/or post-training blockade of 5-HT3 receptors on learning and memory in mice trained in a shock-motivated Y-maze visual discrimination task and a shock-mediated inhibitory avoidance task. Significant improvements in learning and/or memory were seen in both tasks, thus providing evidence for a possible role for 5-HT3 receptors in the mediation of the processes underlying learning and memory in mice trained in these two tasks.

508.12 D-AMPHETAMINE INCREASES STRENGTH BUT DOES NOT DECREASE LATENCY OF SPINAL FIXATION IN RATS. M. J. Bartel, A. G. Heshling*, L. E. Kempe*, J. M. Guadin,* and D.M. Bowser, Department of Psychology and College of Osteopathic Medicine, Ohio Univ. Athens Ohio, 45701

It has been demonstrated that lastind hindlimb flexion (spinal fixation) may be induced by external stimulation to the upper right hindlimb in spinalized rats (Steinmetz et al., J.C.P.P., 95:4, 548-555, 1981). Temporal parameters of spinal fixation were examined by Steinmetz et al. (J.C.P. P., 96:2, 325-327, 1982). In 1989, Bartel et al. (Neurosci. Abst.,15:467, 1989) found d-amphetamine to significantly increase the amount of hindlimb asymmetry. The present study was done to establish the temporal parameters of spinal fixation under d-amphetamine.

In the present study, 70 rats were anesthetized with Nembutal (50 mg/kg) i.p. and randomly assigned to one of seven groups of 10, 20, 25, 30, 40, 45, and 50 minutes stimulation time. Animals were administered d-amphetamine (2mg/kg i.p.) 15 minutes prior to stimulation and then spinalized at T7. Immediately following upper hindlimb stimulation (3-4mA, 100 puls, 700msec, repetitive dc pulses), fixation was measured as the amount of weight needed to remove hindlimb asymmetry.

Results show a generalized increase in the asymmetry for all time groups when compared to results of Steinmetz et al., 1982. with a noted plateau of asymmetry occurring between stimulation times of 30 and 45 minutes. The asymmetry strengthening effect of the d-amphetamine increased with increases in time of stimulation. This work supports earlier work by Bartel et al., 1989, which suggested catacholamine involvement in the fixation. Further work will include a dose-response evaluation of d-amphetamine.

508.15 p-Chloroamphetamine's MEMORY IMPAIRING EFFECTS IN RATS ARE DUE TO SEROTONERGIC RELEASE NOT DEPLETION. A.C. Santucci, R. Gluck*, J. Bangston*, M. Gerard* and V. Haroutunian, Bronx VAMC & Mt. Sinai School of Medicine, New York, NY 10469.

The present investigation examined whether the memory impairing effects of p-chloroamphetamine (PCA) are due to the drug's serotoninergic release or depletion consequences. In Exp. 1, rats received PCA or saline at various intervals before passive avoidance training and were then tested 24 hr later. Results indicated that PCA (2.5 mg/kg) impaired retention relative to saline-injected controls only when the drug was given shortly (15 min - 2 hr) before testing (ps < .05). In Exp. 2, animals were prepared with 5.7 DHT (30 µg/2 µl) DMI pretreatment or sham lesions of the nucleus basalis of Meynert (nBM). Two weeks postoperatively subjects were administered 2.5 mg/kg PCA or saline 30 min prior to passive avoidance training and were then tested 72 hr later. Retention deficits were observed only in rats treated with PCA irrespective of the animal's lesion status (ps < .05). However, both the PCA treatment and the 5.7 DHT lesion procedure produced serotonergic depletions in the hippocampal cortex (44% - 66%) and in the nBM (50% - 85%) (ps < .05). It is concluded that the memory deficits following PCA administration are due to the drug's serotoninergic release effects at sites other than the frontal cortex or the nBM.


We recently reported that post-training administration of serotonergic receptor antagonists attenuated avoidance retention deficits normally exhibited by aged rats. In the present study, we determined whether subeffective doses of a cholinesterase inhibitor, physostigmine, on memory in aged rats using the same task. The drugs were injected ip alone, or in combination, immediately following training. Retention testing occurred 24 hrs following training. A dose-dependent enhancement of memory was demonstrated as a result of the three treatment conditions (i.e., ketanserin, physostigmine, ketanserin + physostigmine). The facilitation of memory produced by the combined treatment was observed at doses well below those required to produce a similar effect when each drug was administered alone. The results provide additional evidence for an interaction between the cholinergic and serotonergic neurotransmitter systems in learning and/or memory, and may have important implications in the treatment of geriatric-related cognitive disorders. (Supported by NIA grant AG07070 and ADRO grant FRG87087).

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
509.1
RIPLEY'S "BELIEVE IT OR NOT!": AN HISTORICAL REPRESENTATION OF NEUROSCIENCE FACTS AND ARTEFACTS IN THE POPULAR PRESS.
E.A. Johnson. Behavioral Neuroscience Program, Department of Psychology, UCLA, Los Angeles, CA 90024.

In 1918, New York sports cartoonist Robert Ripley published the first of what would prove to be 54 volumes of facts and figures under the rubric "Believe It or Not!" (BION). In his worldwide pursuit of truth that was stranger, or at least more interesting, than fiction, Ripley could not help but encounter and advertise some of Neuroscience's more intriguing anecdotes and anecdata. He and his successors presented these in over 300 newspapers in 17 languages, to a circulation reaching at one point 80 million, in the same stylized black and white illustrations and exclusion-pointed statements as his other claims. Ripley's biographers have noted that "his was a mind unalloyed by culture", and that he had "the curiosity of the unlearned". He was interested in the fascinating, the curious, and the bizarre, with often-reprinted neuroscience oddities such as Phineas Gage and trephined skulls falling among the steady stream of topics teasing the public's seemingly insatiable appetite for anecdotes and minutiae. The popular impact of BION is undeniable. BION was a national institution, a forerunner of modern tabloids in the appreciation of the facts of science without consideration of the scientific process, but with a reputation for scrupulous documentation of its claims.

The paper is the beginning of a project examining the popular representation and perception of Neuroscience. Neuroscience-themed BION panels will be presented, compared with their original sources, and examined with respect to salience of interpretation and accuracy of content. Anecdotes of the Annual Meeting will be invited to recollect on their discussions encountering BION and other popular sources, especially as the popular accounts sparked personal interest in the neurosciences.

509.2
H. L. Marshall and H. W. Macon, Brain Research Institute, UCLA, Los Angeles, CA 90024.

The youngest of four brothers who were reared on a frontier midwestern farm that provided the major subsistence for the Baptist minister's family, Charles Judson Herrick assimilated nature, a religious attitude, and a love of learning. He followed his ten-years-older brother, Clarence, to Denison University and the University of Cincinnati and returned to Denison to be associated with him in a new graduate biology program. Charles Judson received his Ph.D. from Columbia University in 1900. After he returned to Denison, he continued a commitment made in 1894 that was of greatest importance to the progress of neuroscience: managing editorship of the Journal of Comparative Neurology, when Clarence became disinterested in 1911. For 30 years he was the journal's editor, setting type when necessary.

Called to the University of Chicago in 1907, Herrick began teaching medical students. He also organized a graduate course that attracted students and faculty from many disciplines. Herrick's writings became prominent after he retired in 1934, the happiest and most fruitful years of his career. His most important publication was The Brain of the Tiger Salander (1948). Not content solely with descriptive research in elegant detail, Herrick explained and amplified his ideas in psychology, to... find out what these animals do with the organs they have and what they do for...

509.3
THE HERRICKS AS NEUROSCIENTISTS I. CLARENCE LUTHER HERRICK 1850-1904.
H. W. Macon and L. H. Marshall, Brain Research Institute, UCLA, Los Angeles, CA 90024.

Clarence Luther Herrick was born near the thriving frontier village of Minneapolis, Minnesota. His interest in nature and intellectual precocity led him, with three adolescent friends, to form the Young Naturalists Society, with serious papers delivered at regular meetings throughout their high school period. By graduation from the University of Minnesota, he had published five articles on natural history topics in scientific journals. During a year's postgraduate study in Leipzig, Herrick translated Rudolf Hermann Lotze's textbook, Outlines of Psychology (1867). As professor of natural history and Denison College in Granville, Ohio, he formalized in his teaching and writing his concepts of psychology, and realized the productive- ness of the ontogeny and phylogenous approach to the brain-mind problem. Three years in Cincinnati saw the peak of his research career and the launch of the Journal of Comparative Neurology. In 1900. An appointment at the new University of Chicago held great hopes that Herrick would be able to form a department integrating interdiscipli- nary studies in neurosciences. The University president, William Rainey Harper, negotiated several promises, however, and Herrick felt he could only resign. When tuberculosis struck, he went to New Mexico and became briefly the first president of its university. He died there at 46 years of age.

509.4
PRESERVED PRIMING OF NOVEL STIMULI IN AMNESIA: EVIDENCE FOR MULTIPLE MEMORY SYSTEMS.
M. E. Smith (1,2) & M. Oscar-Berman (2). I. Psychology, Texas A&M University, College Station, TX, 77843; 2. Boston Univ. School of Medicine. Repetition priming of items with pre-existing codes in memory (e.g. words) might be mediated either by activation of those codes, or by new learning. In contrast, priming of novel stimuli (e.g. pseudowords) almost certainly requires new learning. Consistent with the activation view, studies of perceptual identification (Cermak et al., 1986, Neuro- psychology) and semantic priming (Diamond et al., 1984, J. Abnml. Psychol.) have found sparing of priming of words in amnesia, but impaired priming of pseudowords. In the pre- sent study we measured repetition priming of novel words and pseudowords in a lexical decision task, comparing data from 8 Korsakoff amnesic patients and matched control subjects. We also found priming of words and no priming of pseudowords in amnesia, but only for RT data. The amnesics patients also demonstrated an enhanced tendency to misclassify repeated words as words... This indicates a preserved ability to acquire novel information in amnesia, and is consistent with multiple memory-system theories (e.g. Tulving & Schacter, 1990, Science). Supported by NS07239-06 to MES; CA07112 and NS08209 to MO-B and by the US Dept. of Veteran's Affairs.

509.5
HUNTINGTON'S PATIENTS LEARN MOTOR ASSOCIATIONS, BUT NOT MOTOR SEQUENCES.

The striatum is implicated in motor learning (Heindel, et al., 1989). The present work tested motor learning in Huntington's disease (HD) and normal (ND) subjects. The ND subjects learned all motor skills or only a subset. In Experiment 1 one of four lights appeared, and subjects pushed the appropriate light below the light. Subjects were not told that the sequence of lights repeated. Control subjects were unaware of the sequence, yet showed sequence-specific learning by response times that decreased, then increased when switched to random lights. HD patients did not show sequence-specific learning. Experiment 2 eliminated the repeating sequence and made the stimulation-to-motor association nonspecific. Under these conditions HD patients learned the task normally.

Striatal damage affects tasks requiring association of visual cues and motor responses, but not tasks based on sequences of responses. This dissociation suggests independent subsystems of motor learning. We hypothesize that the striatum sets up rapid, open-loop sequences of movements, possibly through connections with supplementary motor cortex. Additional support, not vision or kinesthesia, guides these movements. The association of visual cues and motor responses operates independently of the striatum, possibly in premotor cortex (Fassingham, 1985).

509.6

A direct recognition version of a delayed non-matching-to-sample task (DMMS) was used to assess visual recognition memory in 9- and 12-month old human infants. The task involved allowing infants to become familiar with a toy, and then recording whether an infant reached for the novel or familiar toy in a pair. Each session included 12 or 18 trials with no delay and either 40, 80, or 200 sec. between familiarization and test. In addition, the delay period was either unfilled or filled with interference caused by removing the toy for 30 sec, or later testing. When the delay period was unfilled, both groups successfully chose the novel toy at the 80 sec. delay interval. However, when interference was introduced during the delay, the 8-month olds performed at chance at the 90 and 200 sec. delays, whereas the 12- month olds performed better than chance at some delay intervals. It is suggested that by 12 months of age, but not by 8 months, the human hippocampus is sufficiently developed to mediate performance across long delay intervals filled with interference.
509.7
FURTHER CHARACTERIZATION OF THE VISUAL PROCESSING DEFICIT IN NEPHROPATHIC CYSTINOSIS. S.N. Nichols*, A.O. Ballantyne*, B.L. Dodge* and P.A. Trauner. Dept. of Neurosciences, Univ. of California-Davis, CA 95616.

Previous studies have demonstrated an isolated deficit in visual processing in children with infantile nephropathic cystinosis, a hereditary lysosomal storage disorder. Children with this disorder appear to have difficulty both with visual memory and with manipulation of mental images. In order to further characterize the problem, 11 children with cystinosis and 11 matched controls were studied using a visual memory task, the Benton Visual Retention Test (VRT).

Cystinotic subjects made significantly more errors involving reproduction of internal details of figures on the VRT than did controls (1.9±1.6 vs. 0.9±1.1, p=0.02). The 2 groups did not differ significantly in their tendency to distort the overall shape of the figures. Preliminary analyses of 2 other tasks, Collin Incomplete Figures and Closure Speed, suggest that cystinotics may perform more poorly on tests involving identification of incomplete figures, a skill requiring integration of detail.

These data suggest that children with cystinosis may be impaired in their ability to maintain details of an image in memory or to form an accurate mental image when incomplete visual information is presented.

509.9
NORMAL ORGANIZATION OF CATEGORY KNOWLEDGE IN ALZHEIMER'S DISEASE. A. Cronin-Golomb, A. Kokolos*, S. Carkin, J.H. Growdon. Dept. of Brain and Cognitive Sciences, MIT, Cambridge MA 02139, Dept. of Psychology, Boston University, MA 02215, and Dept. of Neurology, Massachusetts General Hospital, Boston MA 02114.

Deficits in category knowledge in Alzheimer's disease (AD) may be attributable to a disruption in underlying organization, or to impaired retrieval of properly organized information. We addressed these alternative possibilities with 3 tasks: (1) category decision (Is a member of category?); (2) ranking of category exemplars by typicality (e.g., apple - orange - banana - 'typical' vs. apple - banana - 'atypical' types of vehicles); and (3) fluency (name as many members of category as possible in 1 minute). Comparisons of 18 patients with AD, who varied in dementia severity, and 14 age-matched control subjects (CS) revealed highly similar patterns of performance. Certain of the 10 categories proved more difficult than others in all 3 tasks. The correlation between AD and CS performance was 0.83 for Task 1, 0.88 for Task 2, and 0.98 for Task 3. The pattern similarity existed despite other group differences, e.g., slower reaction times for AD than CS in Task 1, and production of fewer items by AD than CS for Task 3. The results suggest that, although retrieval of category information is impaired in AD, the underlying organization of category knowledge is normal.

509.11

Although there is clear evidence that the brain is a primary site of human immunodeficiency virus (HIV) infection, controversy exists concerning whether the early presence of HIV is associated with any concomitant behavioral or cognitive dysfunction. Furthermore, although evidence for the presence of HIV is now being presented, diagnostic criteria are in need of further study. In this preliminary report we present three cases of such a deficit, manifesting as an alteration in the ability to produce a meaningful temporal sequence of events as elicited through standard neuropsychological assessment. In this report we present three cases of such a deficit, manifesting as an alteration in the ability to produce a meaningful temporal sequence of events as elicited through standard neuropsychological assessment. In this preliminary report we present three cases of such a deficit, manifesting as an alteration in the ability to produce a meaningful temporal sequence of events as elicited through standard neuropsychological assessment.

509.8
PROCESSING OF SPATIAL RELATIONS WITHIN AND BETWEEN THE DISCONNECTED CEREBRAL HEMISPHERES. J. Sergent, Montreal Neurological Institute, McGill University, Montreal, Canada.

This study examined 1 issues related to the processing of spatial relations by commissurotized subjects. 1. The respective competence of the disconnected hemispheres at performing judgments of relative position and distance between two objects was tested. The results showed that the two hemispheres were equally competent at representing and operating on these spatial relations even with different representations, and the capacity of the disconnected hemispheres to operate conjointly was then examined. The two disconnected hemispheres were simultaneously stimulated with the same information to produce a single response based on this information. Compared to unilateral stimulations, bilateral stimulations resulted in enhanced response accuracy and, depending on the type of task, in enhanced response latency and accuracy different from the patterns of either unilateral condition. Results of this study suggest that the two disconnected hemispheres can operate simultaneously and are able to join the outcomes of their respective operations before the production of a single response. 2. Interhemispheric communication. Additional information was studied. Unlike pattern information that is typically confined to the hemisphere that receives it in the commissurotized brain, visuoperceptual information could be subjected to interhemispheric comparison as a function of its categorical and metric properties, although the patients had only implicit knowledge of part of the transferred information.

509.10
COGNITIVE ABILITIES IN LESBIANS. C.W. McCormick and S.P. Witelson, Departments of Psychology and Psychiatry, McMaster University, Hamilton, Canada.

We previously reported an increased prevalence of non right-hand preference in homosexual women and interpreted this result as evidence that atypical cerebral asymmetry is a factor in homosexuality (McCormick et al., 1987, 1990). In the present study, we compared homosexual females to heterosexual females and males (n = 31 per group), matched for age, handedness and cognitive ability tests that typically reveal differences between males and females (spatial ability, fluency). Previous results with homosexual males (McCormick & McCrackin, 1989) indicated lower spatial ability and higher fluency in homosexual males than in heterosexual males. However, among non right-handed subjects, homosexual females scored lower on cognitive tests than did heterosexual females. These results are examined with reference to neuropsychological studies of women exposed to atypical levels of prenatal hormones.

509.12
LAUGHTER: SOCIAL CONTEXT, STRUCTURE, AND CONTAGION. R.R. Provins. Dept. of Psychology, Univ. of Maryland Baltimore County, Baltimore, MD 21228.

Laughter is an ubiquitous, species-typical human signal and motor act. Laughter is almost exclusively social, even more so than smiling and talking; it occurs 30 times more frequently in social than in solitary settings. Laughter is characterized by stereotyped laugh note duration ("ha") and inter-note-interval ("ha-ha-ha-ha-"), and is often accompanied by bodily movements. Laughter can be induced by music ("laughter"), laughter by" laughter" of this kind is known and has even spawned the technology of the "laugh track" on broadcast comedy shows, the ramifications of which are discussed. Contagous laughter, however, is not contagious in the everyday sense of the word. Contagious laughter has not been appreciated. The potent contagious laughter effect suggests that humans have evolved a neurophysiological mechanism to detect laughter when triggered by a stimulus having the unique stimulus properties of laughter. The stereotype of laughter as an acoustic stimulus and motor act and the presence of the contagious laughter effect suggests that modular mechanisms are involved in both laugh production and reception. These data provide insights into the curious behavior of laughter, the evolution of language, and a variety of issues in cognitive neuroscience.

A well-substantiated fact that is partially explained by drugs, toxic elements, environmental stress, illness, emotional strain, and nutritional deficits. Scientific study of these factors is influenced by the availability of suitable metrics. In a series of experiments, various treatments (e.g., halon, hydrocortisone, hypoxia, alcohol) and holistic measures of mental acuity (WAIS, ASVAB, Wonderlic, ACT) were given to various groups of subjects. An Automated Performance Tests System (APTS) battery of repeated-measures cognitive, perceptual, information-processing, and motor tests was administered in standardized format in all the studies. Using APTS scores as predictors, regression equations were calculated to determine performance impairment as a function of alcohol dosage (the criterion) in one carefully graded experiment. Performance decrement was correlated with blood alcohol level (r = .88) using a composite APTS score. Regression equations were then calculated for these same tests using the holistic intelligence measures. Then the dozen sensitivity studies, using various drugs, were translated into the effects that those agents had on mental activity using his battery regression equations. The ability to use such a dose equivalence method to conduct human performance bioassays is discussed as it relates to neuroscience research and for practical workplace decisions.

509.15

ELECTROCARDIOGRAM COHERENCE AND CORRELATION OF AMPLITUDE MODULATION BETWEEN ELECTRODES EIGHT DISCRETE IN MILLIMETERS IN HUMANS AS WELL AS IN RABBIT BRAINS. T. L. Bublock, V. I. Irpin* and J. P. Alles*, Dept. of Neuroscience, U.C.S.C.D., La Jolla, CA 92037.

Coherence is not typically widespread over the cortex; it may have a significant microstructure. Metal disk electrodes 5-10 mm apart, embedded in plastic strips, inserted under local bar on frontal, parietal and temporal lobes of epiloid patients recorded electrocardiogram (ECG) during sleep, wakefulness and seizure. We computed coherence (Coh) for frequencies (F) 1-80 Hz between all 130 pairs among 16 electrodes plotted Coh vs distance (D) between electrodes, pooling lobes but not states or patients. Averaging over many pairs, Coh declines monotonically with increasing D, for all F. Coh vs F shows no prominent patterns in Coh with F are commonly slight. Though widely different among subjects and electrode sites, a roughly median value of 5-15 mm for Coh vs F in humans compared with 2-5.5 mm reported for rabbits (T.H. Bublock, A.C. McClune ERG Clin. Neurophysiol. 72:79-80, 1999). This is probably the best measure of the degree of synchronization in domains of cortex. Between slow-wave sleep and alert states differences in these values are large. Adenosine, a feature, quite distinct from Coh, is the coherence between electrode loci of the amplitude modulation (A) of narrow ECG bands. For 6 bands: 1-4, 4-8, 8-13, 13-15, 19-30, 30-45 Hz, we computed AM envelopes, low-passed those to 4 Hz, correlated (AMCor) each pair of electrodes, and plotted this value vs D. AMCor declines with D; at 10 mm mean value is usually 0.4-6.0 for all F's, slightly more for the lowest; at 20 mm 0.3-0.4; at 30 mm 0 correlations are just significantly negative. It remains to learn whether these two aspects of cooperation vary with state or region.

509.17


The goal of neuromagnetic recording techniques is high resolution mapping of head brain activity. This goal has led to the discovery of an ill-posed inverse problem; namely, the determination of the number, location, spatial configuration, strength, and timecourse of the neuronal currents that give rise to the magnetic field distribution at the head surface. To help resolve ambiguities in the source modeling process, we are using a system for anatomical segmentation and volumetric reconstruction based on MR images (George et al., Magnetic Resonance Imaging, 1990). To investigate alternative strategies for constraining the locations of allowable sources. One strategy uses proximity to the local cortical surface to generate constraints. A second strategy incorporates an a priori standard least-squares minimization algorithm. Another strategy makes use of the fact that surface magnetic fields are a nonlinear function of location but a linear function of source orientation and current strength. Field distributions may therefore be expressed as linear combinations over a basis matrix including field amplitudes at each sensor location for unit current vectors in x, y, and z dimensions. A constrained basis matrix formulation can be combined with pseudo-inverse procedures such as the minimum norm lead field expansion to increase the anatomical realism of mathematical reconstruction procedures.

509.18


A critical step in attempting to model the surface magnetic fields generated by the human brain is determining the number of sources (i.e., the "order" of the model). To address this question we conducted extensive simulations of point current dipole sources in a homogeneous spherical conductive medium in which the number, spatial configuration and strength of sources and amount of noise varied. Three approaches to the identification of model order from the simulated instantaneous magnetic field distributions were evaluated: (1) visual inspection of model fit, (2) comparison of percent variance accounted for by single- versus multi-source models; and (3) examination of the reduced chi-square values (Chi-square degrees-of-freedom) for alternative models. When the true noise level is known, the reduced chi-square approach provides the most sensitive and valid means for determining model order. However, this method is sensitive to both the accuracy of noise estimates (for empirical data) and the magnitude of the noise. For example, when the noise level is high, a chi-square test may indicate that a single source configuration could have generated a field pattern actually produced by multiple dipoles. This approach has been explored for empirical magnetic data elicited by the simultaneous and separate presentation of visual stimuli.
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

S.1.0 EFFECTS OF FOREBRAIN LESIONS ON DRINKING AND SALT APPETITE AFTER DOCA OR YOHIMBINE. D.A. Fitts, Dept. of Psychology, Univ. of Washington, Seattle, WA 98195.

Lesions of the ventral part of the ventral median preoptic nucleus (VMNmPO) enhanced daily salt appetite induced by ic injections of deoxycorticosterone acetate (DOCA), but had no effect on acute salt appetite or water intake after sc injection of 5 mg/kg yohimbine (yohimbine: method: Johnih... et al. Soc. Neurosci. Abstr. 15, 964, 1989.) Lesions of the subolfactory organizing center (OSOC) did not affect on daily saline or water intakes during 3 mg/kg/day DOCA, but significantly reduced acute water intake after yohimbine. Pretreatment with a low dose of 100 mg/kg captopril ip did not significantly reduce either the water or saline intakes after yohimbine injections in normal rats, so these behaviors do not appear to depend on peripheral angiotensin synthesis. The findings demonstrate that the enhanced DOCA-induced saline intake in VMNmPO-lesioned rats is specific to lesions of the ventral lamina terminals. By contrast, the VMNmPO lesion reduces salt appetite after angiotensin-related treatments such as captopril or sodium depletion, and has not been found to affect stimulated water intake (Fitts, et al. Behav. Neurosci., in press). SFO lesions do not affect salt appetite in daily tests, but do reduce acute or chronic water intake after treatments such as angiotensin, captopril or yohimbine. Supported by NS2274.

S.1.0.3 SODIUM SENSITIVITY AND ORIGINS OF THE PRIMARY POLYPYDYSIS OF THE INBRED MICE. K. Koizumi, K. Iinuma*, N. Akamato* and H. Yamasaki. Dept of Physiology, SUNY Health Science Center and School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan.

The excessive drinking (5-8 times that of normals) of the inbred mice, ST/N, which is not due to hypoglycemia or renal dysfunction, was reduced to 35% of control level by low Na* diet (10% of normal food). Reduction in water intake began on Day-1 and continued for 9 days after the end of low Na* diet which was given for 18 days. In non-polydipsic S/W mice, water intake was reduced to 60% of the control from Day-10 to Day-18 of NaCl depletions. Water intake was not much affected in both strains. Since osmolality and Na* concentration of plasma are similar in both strains, the results suggest that the ST/N mice require NaCl for daily water intake. To test this hypothesis, in brain slice preparations responses of the AV3V-OLT neurons to changes in osmolality and Na* concentration of the bathing medium were studied using extracellular recordings. No significant differences in their responses were found between the ST/N and S/W. In both groups the neurons were inhibited by hyperosmotic and excited by hypotonic saline. Replacing Na* with mannitol or sucrose did not alter these responses, indicating that no difference existed in sensitivity of the neurons of both strains to Na*. Our findings suggest that neurons located in the CNS other than in the AV3V may be involved in altered Na* sensitivity, or that other mechanisms play a role in the observed attenuation of the polydipsia by low Na* diet. (Supported in part by a grant from USPHS, NS-00847).


The structures of the AV3V are rich in angiotensin (ANG) binding sites and may therefore be important for the participation of central ANG, ALD or DOCA (ALDO) in the AV3V. The results of the present study suggest that these neurons are involved in the regulation of fluid intake and renal sodium balance. The AV3V-OLT neurons may be involved in the regulation of fluid intake and renal sodium balance.


The hepatic branch of the vagus nerve has been suggested to be necessary for the normal satiation of dependence-induced NaCl intake (Toftoff, 1986, 1997). If this is true, removal of the hepatic vagus should result in 1) an increase in NaCl intake and 2) less decrease in NaCl intake when preloaded with NaCl. To test this, depleased naive, male, Long-Evans rats were given either sham (n = 12) or hepatic (n = 12) vagotomies. Following recovery, all rats were sodium depleted by Laxos (furosemide, 10 mg/kg) and overnight sodium-depleted diet and water. At 18 h later, half of each surgical group received no load (NL) or a load (L) of 7.5 ml of 0.15 M NaCl in the stomach. Ninety min later, the rats were offered 0.3 M NaCl and water in 1-h NaCl-appetite test.

At the end of the first 35 min, the hepatic vagotomized group showed a normal NaCl intake (NL/15 min = 5.6±1.1 ml vs 7.4±1.9 ml) and a normal suppression of NaCl intake to the preload of NaCl (H/L = 3.3±1.0 ml vs 5.5±1.0 ml), but there was no effect of surgical group (F(1, 20) = 0.29, p = 0.60). At 60 min, there were still no significant differences between the H/L (4.0±1.2 ml) and S/L (3.0±0.9 ml) groups. The H/L (5.5±0.85 ml) and S/L (3.9±0.95 ml) groups were not significantly different (F(1,10) = 3.18, p = 0.11), although the intake from 15-60 min was 46.4% less for the H group.

If the hepatic vagotomized rats had a more rapid emptying of the NaCl preload, this could obscure a satiety deficit. To test this, the rats were sodium depleted again and 90 min after the NaCl preload (1.48 ml), they were anesthetized and their stomachs were removed. The stomach contents were measured and the sodium concentrations were determined by flame photometry. There were no differences in the residual stomach sodium content (mEq) between the two groups (S = 0.117±0.004, H = 0.096±0.042). Thus, under our conditions, the hepatic vagus was not necessary for a normal satiation of NaCl appetite.

Supported by NIH DK-39810 (SPF) and NIH MH-00149 & MH-15455 (GPS).

S.1.0.4 SODIUM APPETITE IN RATS AFTER DIETARY SODIUM DEPRIVATION: INHIBITION BY ESTROGEN. E.M. Stricker, E. Thiele and J.G. Verbalis. Dept. of Behavioral Neuroscience, College of Arts and Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

Little sodium appetite is observed when rats are deprived of dietary Na for 4 days, presumably because aldosterone secretion minimizes renal Na losses. When Na deprivation is extended to 8 days, however, a sodium deprivation appetite results in adult male rats that far exceeds their urinary Na losses during the deprivation period; in the intact rat, 19.0±2.8 M Na was excreted in 7 hr, which is as much NaCl as rats have ever been reported to drink rapidly. In contrast, female rats drank far less NaCl (2.6±0.7 M NaCl) after 8 days of Na deprivation (8±1±7 hr). Because of this sexual dimorphism in sodium appetite, we also studied Na-depleted male rats in gonadectomized form after 8 days of Na deprivation. Both male and female animals drank comparable amounts of saline as intact males but reverted to the low intakes of intact females when replaced with physiologic amounts of estrogen. These results indicate that a robust sodium appetite can be predisposed in rats by extended dietary Na deprivation, and that estrogen has a marked inhibitory effect on the induced sodium appetite under these conditions.

S.1.0.6 THE REVERSAL OF THE SODIUM CHLORIDE AVersion OF FISCHER-344 RATS BY CHORDA TYPHAMI NERVE TRANSSECTION. G. Lollis* and R. Bernstein. Dept. of Psychology, University of Washington, Seattle, WA 98195.

Fischer-344 (F-344) rats are atypical in their lack of a preference for any concentration of NaCl solution and their avoidance of NaCl solutions preferred by other rat strains. The chorda tympani (CT) indicate greater responsiveness of the CT to NaCl stimulation in F-344 rats than in other strains, such as Wistar. Moreover, the exaggerated CT response appears to be associated with greater sensitivity to the sodium channel blocker amiloride. This suggests that when F-344 rats drink NaCl solutions amplified signals from the CT may contribute importantly to their NaCl aversion. The present experiments were designed to determine whether the F-344 rat's NaCl aversion persists after bilateral transection of the CT nerve. In adult male F-344 rats the chorda tympani was sectioned bilaterally (CTX) or was exposed but not sectioned (SHAM). The present experiments were given to CTX and SHAM rats beginning two weeks after surgery. Concentrations of NaCl (0.6%, 0.8%, 1.0%) maximally preferred by other strains yet avoided by intact Fischer-344. Ninety min after the oral concentrations tested, CTX animals preferred NaCl solutions to water. This preference differed dramatically from the avoidance of these solutions by SHAM animals. CT cuts to the chorda tympani have not significantly affected NaCl preference. This CTX animals preferred NaCl solutions, rather than just failed to avoid them, indicates that they continue to detect the taste of NaCl after CT transection. These findings are consistent with the hypothesis that the F-344 rat's aversion to the taste of NaCl stems from input provided by the CT nerve, particular that component of the CT response which is sensitive to amiloride.

The central nucleus of the amygdala is the end-station in the ventral forebrain for taste visceral afferents and it is rich in angiotensin-containing terminals. It, therefore, may be an important neural network that mediates the angiotensin/aldosterone synergy that underlies increased NaCl intake in the rat. Accordingly, the 30Na/Cl and water intake of rats with bilateral ablation of the central nucleus of the amygdala were studied. After strenuous postoperative recovery of food and water intake, 1) daily water intake of NaCl-saline treated rats was increased by 7 days compared to the sham-operated controls, 2) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 3) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 4) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 5) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 6) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 7) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 8) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 9) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 10) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 11) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 12) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 13) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 14) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 15) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 16) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 17) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 18) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 19) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 20) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 21) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 22) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 23) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 24) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 25) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 26) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 27) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 28) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 29) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls, 30) the NaCl intake of NaCl saline rats was increased by 7 days compared to the sham-operated controls.

CENTRAL ANGIOTENIN II (ANG II) RECEPTOR BLOCKADE REDUCES YOHIMBINE-INDUCED SALT APPETITE. B.L. Thuborost, K.M. Higgins & A.K. Johnson. Univ. of Iowa. Iowa City, IA 52242

Subcutaneous (s.c.) injection of the alpha-2 adrenergic antagonist yohimbine (YOH) rapidly produces salt appetite in sodium-deprived rats. This effect produces a substantial increase in mineralocorticoid production, thus showing independence from the peripheral renin-angiotensin system. We tested for a role of central ANG II receptors by using chronic bilateral kainate lesions of the lateral (with 8% NaCl) or the medial (with saline) side of the brain. These lesions produced a significant decrease in water intake and a significant increase in salt intake. Therefore, the central ANG II receptors may play an important role in the control of NaCl appetite in the rat.

THURSDAY PM

INGESTIVE BEHAVIOR: SALTS, WATER AND AVERTION

13845

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

13845

13845
510.13

Rats' intakes and 2-bottle preferences for sucrose and QNC varied over the 12/12 LD cycle (Dracos & Flynn, 1991). Longitudinal experiments were run in one room to study the role of oral from postoral effects on diurnal preference differences. Rats were entrained to a 12/12 LD cycle and fitted with intracerebroventricular (ICV) cannulae, either the Light (Group, n=5) or dark Group (n=4), were rats were presented with 4 ascending concentrations of sucrose (0.0125%, 0.025%, 0.05%, and 0.1% QNC) and allowed to drink for 30 min on 1 h. The Dark Group drank significantly more 0.3 M sucrose (78.7 ± 12.4 ml) and 1.0 M sucrose (0.0 ± 7.0 ml) in 1 h than did rats in the Light Group (40.4 ± 5.9 ml and 41.6 ± 11.0 ml respectively), p<0.05. In comparison to the Light Group, the Dark Group drank significantly more 0.0 M NaCl but significantly less of 0.3 M, 0.1 M, and 0.3 M NaCl, p<0.05. There were no group differences in response to HCl. The Dark Group drank more 3 x 10–3 M QNC than the Light Group, p<0.05. These results demonstrate that rats' intakes are influenced by the changes in physiology that accompany the 12/12 LD cycle and suggest that taste is affected by the diurnal cycle. (Supported by NE-24879 awarded to F. W. Flynn.)

510.14
DIPSODIC STIMULI PAIRED WITH EATING SUPPORT CONDITIONED INITIATION OF DRINKING WITHOUT CONDITIONED WATER INTAKE IN RATS. E. D. Braly and K. A. Siqueland. Dept. of Psychology, Colgate University, Clark 103.

The claim (Fitzsimmons & Leffman, JCPP 67:273, 1976) that rats learn to associate peripherally food properties of fruit with postprandial dehydration consequences remains unexamined. We have done experiments in which an ingested food conditioned stimulus (CS) is paired with one of various dipsodic unconditioned stimuli (UCS) such as 20 mg/kg lithium chloride (LiCl), 0.9% NaCl or 0.9% NaCl as a control known to be putative signals for drinking allowed by injected LiCl. The results of the UCS pairings on 2, 6 or 9 trials were followed by an extinction trial (i.e., CS without UCS) to examine for conditioned drinking. With a 10-min-US-UCS interval rats conditioned a suppression (p<.001) to a liquid food CS without evidence (p>.10) for conditioned drinking the histamine UCS supported neither conditioned aversion nor water intake (p>.20). With a 10-min-US-US interval the IM NaCl and histamine UCS both supported conditioned inhibition of drinking, measured by shorter (p<.05) latency to initiate drinking upon presentation of CS on the extinction trial (vs. baseline) without supporting conditioned water intakes. These results suggest that (a) putative signals for food-related drinking, cell dehydration and histamine, serve as UCS for conditioned alimentary experiences, the present experiment determined if pica shows a systematic relationship to the amount of food withheld. Accordingly, rats maintained with water and clay (kaolin) continuously available were subjected to periodic episodes of food restriction in a counterbalanced Latin square design. Thirty animals that had consumed at least 15 g of kaolin in 24 h together with the group receiving 20 mg/kg LiCl were used for 20 trials. The results indicate that pica induced by food deprivation is similar to other causes of pica and that it is mediated by subjective visceral sensations.

510.15

In 6-day-old rat pups, both dehydration and overnight deprivation enhance ingestion. The modulatory effects of dehydration and deprivation may have similar neural bases, or may be accomplished by different neural mechanisms. To examine these issues, we studied the neurochemistry of neural metabolic activity during a 1 hour (14[2]C] 2-deoxyglucose (30 uCi/100g BW, s.c.) incorporation period was compared in 6-day-old rat pups that were celluly bathed by placing them in a chamber that were 24 hours or 24 hours deprived. Autoradiographic images corresponding to sections of the neural areas at several levels of the hindbrain were selected for each experimental group and analyzed. Difference images were then generated by subtracting average images from one another. Relative to 24- hour deprived pups, celluly dehydrated pups showed increased metabolic activity in para-britocel rhinal nucleus, nucleus of the mesencephalic trigeminal nerve, oral regions of the nucleus of the spinal trigeminal nerve, nucleus ambiguous, and areas of pons and pontine reticular areas.

510.16

Geophagy (clay consumption) is a common form of pica (consumption of nonnutritive substances) during famines. Rats will frequently engage in appreciable amounts of pica during brief periods (24 h) of food deprivation. Previous experiments employing emetic toxins, motion sickness, and conditioned illnesses indicate that pica relieves the subjective aversive symptoms of gastrointestinal malaise. Assuming that over short deprivation periods the more food withheld, the more severe or prolonged the unpleasant gastrointestinal sensations, the present experiment determined if pica shows a systematic relationship to the amount of food withheld. Accordingly, rats were tested in a unique sequence of conditions spaced 6 days apart during which they were permitted a 12 h access to 0, 5, 10, 15 20 g or ad lib food. They consumed 19.9, 10.1, 7.6, 5.3, 3.2, and 0.2 g of kaolin respectively. These results indicate that pica induced by food deprivation is similar to other causes of pica and that it is mediated by subjective visceral sensations.

510.17

Previously (Pharm Biochem & Behav 35:359, 1985) we found semi-purified (10-20uM) k-SAT produced a two day conditioned taste aversion in a two-bottle choice after ICV infusion. In the present study sp-SAT was purified on HPLC which yielded two peaks (P-A, 10.7%; P-B, 89.3% by wt). Male Sprague Dawley rats (327-460g) were fitted with chronic in-CL (L3132, light out at 1131h) were given access to water in calibrated bottles, for 1 hr (100-1130h) and food ad libium for 4 weeks. The rats were then divided into three groups (GRP); GRP 1, control (n=8); GRP 2, P-A, 11µg/kg, (P-B, 1-10µg/kg, P-B, 1-10µg/kg. On day 1 all groups were ICV infused (10µl) with artificial-crebrospinal fluid (a-CSF) at 1000 h. At 1000 h, the control group was given almond flavored (0.5% extract) water (AFW) and the experimental groups banana flavored water (BFW). One hour and 24 h food intake (FI) and 1 h fluid intake were measured and found to be similar in all groups. On the next day the groups were ICV infused with: GRP 1, a-CSF and given BFW, GRP 2, P-A, 11µg/kg and GRP 2, P-B, 90µg/kg, both groups were given AFW. Fluid intake was similar in all groups, however, 1 h fluid FI were both groups (14±3.8, 11±2.8% compared to GRP 1 (2.6±1.5, 16±1±1%) and GRP 2 (3.0±1.5, 14.8±1.2%) on day 4 the groups were given a two bottle choice test of AFW vs BFW, GRP 1, 7.1±2.11±11±14±4 mice, GRP 2, 6.9±8.9±8.9±13 mice, GRP 3, 9.2±17.7±9.2±18 mice, all N=5. These data suggest HPLC purified P-B can suppress FI without producing taste aversion and thus may be a physiological probe.

Supported in part by NIH-DAK2653 and Baylor University Funds.

510.18

Lithium has been shown to be a highly effective agent in inducing conditioned food aversions (CFA). The effects of chronic LiCl infusion on food intake and diet preference in rats with area postrema lesions (APx) or sham lesion (APx), were examined. Osmotic minipumps, filled with a saturated aqueous solution of LiCl, were implanted into the perioral cavity of APx and APx rats. Similarly, nonfunctional pumps were implanted in two other groups of APx and APx rats. The LiCl infusion or sham drug phases were paired with free access to a novel food item in the non-conditioning phase. Food intake was monitored during the conditioning phase and preferences for the novel diet vs. a familiar diet were assessed a week after the conditioning phase. During the conditioning phase the APx groups infused with LiCl exhibited a significant reduction in food intake relative to the sham drug APx group (p<.01). No significant differences in food consumption were found between the two APx groups. After the LiCl injections, animals showed a marked aversion to the novel diet, whereas group APx group given LiCl did not. These results indicate that CFAs induced with chronic LiCl infusions are mediated by the area postrema. (Supported by a NSERC grant to KPO).
510.19


The 5HT-3 antagonist zacopride and the 5HT-1a agonist 8-OH-DPAT have both been shown to possess anxiolytic properties. Two separate experiments examined the effects of zacopride and 8-OH-DPAT on body rotation-induced conditioned taste aversions (CTA) in male hooded rats. The rats were adapted to a 23.5 h/day water deprivation schedule. On 3 conditioning days 30 min access to a 0.1% sodium saccharin solution was followed by subcutaneous administration of either zacopride (0.1 mg/kg), 8-OH-DPAT (0.1 mg/kg), or isonicotic saline. Fifteen minutes following drug administration the animals were exposed to the body rotation procedure which consisted of 30 min rotation at 70 rpm on a schedule of 60 s on - 60 s off. The animals were again given access to water (30 min) every 2 h. On each of the next 4 days and were then tested for saccharin preference (two-bottle tests) over the next 12 days. The results indicated that administration of 8-OH-DPAT significantly enhanced the body rotation-induced CTA (p < .01), whereas zacopride significantly attenuated the induced CTA (p < .05). (Supported by a grant from NSERC to K.P.O.)

510.20

IBOTENIC ACID LESIONS OF THE LATERAL HYPOTHALAMUS BLUNT AFFECT BUT DO NOT INDUCE TASTE AVERSION. K.C. Berridge, The University of Michigan, Department of Psychology, Ann Arbor, MI, 49109.

Aphagia and increased aversive taste reactivity (gapes, etc.) occur after electrolytic lateral hypothalamic lesions (Teitelbaum & Epstein, '62; Schuller & Whishaw, '78; Stellar et al., '79; Flaherty & Grill, '81) and after excitotoxic striatopallidal lesions (Berridge & Cromwell, in press). Aversion is not enhanced, in contrast, after nigrostriatal 6-OHDA lesions that produce aphagia (Berridge et al., '89). Do excitotoxic lesions of the lateral hypothalamus that produce aphagia increase aversion to tastes? Thirty-two male rats received bilateral injections of ibotenic acid (0.1 ul, 1.2 M) into the lateral hypothalamus (A-2.0, L±1.9, V-8.0). Ten rats showed aphagia that lasted at least 8 days after the lesion. Nine control rats received vehicle injections. Aversion to 1 ml oral infusions of sucrose, NaCl, citric acid, and quinine solutions (delivered via chronic oral cannulae) was videotaped and assessed frame-by-frame.

Aphagic rats showed reduced levels of aversive and hedonic reactions (tongue protrusions, etc) to tastes, compared to controls rats or to rats that were not aphagic after lesions. Although a reduction of sensorimotor arousal might explain this result, sensorimotor deficits have been argued not to be produced by excitotoxic hypothalamic lesions (Dunnett, et al., '85). An alternative explanation is that a global blunting of hedonic and aversive affect is produced by the loss of intrinsic neurons from the lateral hypothalamus. Enhancement of aversion by electrolytic lesions may require a synergistic destruction of multiple hypothalamic elements. Alternatively, enhanced aversion may require destruction of neurons from bordering regions that are outside of the hypothalamus itself.

510.21

TASTE AVERSION LEARNING IN THE FERRET: LIMITS ON CONDITIONED AND UNCONDITIONED STIMULI. B. M. Rabin and V. A. Hunt*. Armed Forces Radiobiology Research Institute, Bethesda, MD 20814, and University of Maryland Baltimore County, Baltimore, MD 21228.

Previous research has shown that treatment with toxic stimuli that produces vomiting also produces conditioned taste aversion (CTA) learning at a lower dose. To further evaluate the relationship between emesis and CTA learning, ferrets were allowed to drink a 10% sucrose solution immediately prior to injection of lithium chloride (LiCl) or exposure to ionizing radiation. Treatment with either unconditioned stimulus resulted in the acquisition of a CTA, even when vomiting was produced by the toxin. In contrast, when canned cat food was used as the conditioned stimulus, a CTA was produced by injection of LiCl (3.0 mEq/kg), but not by exposure to radiation (200 cGy). These results, in relation to those of other studies, indicate that the association between the CTA and emetic effects of exposure to toxins may be species specific, depending upon the nature of both conditioned and unconditioned stimuli.

511.1

A NEW METHOD FOR ESTIMATING SPATIAL LOCALIZATION IN RATS. A. Spekman* & J. O'Keefe*. (Spon: Brain Research Association) Anatomy Dept, University College, London WC1E 6BT UK.

A widely used apparatus for estimating spatial localization in studies of hippocampal function is the 'water maze' (Morris, R.G.M., Learn. Motiv., 1981, 12:239). We describe a task which is similar in principle, but which has the advantage that the rat is not immersed in water. Instead the rat is motivated by appetite to approach a goal defined only by its spatial relationship to distal cues.

A small skull-mounted post is chronically implanted in a rat. During training an apparatus is attached with a nozzle which sits in front of the animal's mouth during free behavioural movements around a circular arena (1.18m diameter). A length of plastic tube connects the dispensing apparatus to a distant solenoid valve and pressurised liquid reservoir. Operation of the solenoid valve results in a squirt of saccharine solution directly into the animal's mouth. Typically a volume of 0.1 ml saccharine at a concentration of 0.4% is dispensed over 100ms. A computer is used to track the animal's position and to administer the reward at particular places (goals).

We have shown that rats will learn to approach goals defined in this manner. In addition, by rotating controlled cues from trial to trial, we have shown that distal cues can control spatial localization of this type. Two methods have been used to gauge the accuracy of spatial localization in relation to the cues. In a 'translation' method, rats received reward over a particular spatial area which gradually shrunk/expanded as the rat demonstrated it could/could not find the goal. In the 'radial reward' method, the probability density of reward varied as an inverse function of the distance from the goal.

511.2


To study the role of thalamic lesions in DNMTS deficits caused by pyrithione administration (NiCl₂ Abst. 15:304, 1989), 48 rats were pretrained on DNMTS and given one of four treatments: midline thalamic lesion, (N=16) bilateral thalamic lesion (±1 mm from midline), (N=16), sham lesion of hippocampus overlying the bilateral site (N=8), and sham surgery (N=8). The injections of 5ul of 1 ml NMDA destroyed tissue within a radius of .45 mm of the cannula. None of the lesioned animals were impaired on measures of response accuracy or speed at any of the memory delays tested. These data contrast with those of more extensive RF lesions of the thalamus (cf. Hair, et al, this meeting).
511.3 Diencephalic Anterograde Amnesia for Spatial Information in the Rat. R. J. Sutherland, H. N. Rice* and J. M. Hoeting, Dept. of Psychology, The Univ. of Lethbridge, Lethbridge, AB, Canada, T1K 3M1.

The necessary subcortical damage for "diencephalic amnesia" is not established. We sought to determine: 1. if preoperative "procedural" aspects of the Morris water task would amolter the deficit in new place learning associated with thalamic or mammillary damage and 2. the relative magnitude of impairment associated with damage to anterior thalamic (AT), mediadorsal thalamic (MD), and/or mammillary bodies (MB). All rats were retested in a new platform in the Morris water task. Each day the platform was positioned in a new location in the maze. They were divided into 5 groups receiving electrolytic lesions to MB, AT, MB+MD, or sham lesions. All rats were retested in the moving platform version of the Morris water task. Neither MB nor MB damage alone reliably impaired performance. Rats with AT or MD+MB damage showed an inability to learn the hidden platform location throughout postoperative testing. The results provide support for the idea that combined damage to circuitry involving MD and MB is necessary to produce a severe diencephalic anterograde amnesia. Interestingly, performance in this task is not sensitive to any other damage.

511.5 Impaired Spatial Memory Following Ventromedial Thalamic Lesions in Rats. J. D. Butler and D. B. Neill, Dept. of Psychology, Emory University, Atlanta, GA 30322.

Neuroanatomical and electrophysiological experiments indicate that the ventromedial nucleus of the thalamus (VMT) may have a specific temporal pattern of thalamic lesions revealed an in the acquisition, but not the retention, of the number of conditioned responses. In the present experiment, we evaluated the effects of VMT lesions by using a commonly-used test of "working" memory, the radial arm maze. Ten naive, male Sprague-Dawley rats were trained to retrieve food from the arms of a 8-arm radial maze. Each daily test consisted of a study phase in which four arms of the maze were blocked. The rats retrieved food from the non-blocked arms and were then removed from the maze for 45 sec. During this interval, the remaining arms of the maze were opened. Upon reinsertion to the maze (retention phase), the task was to retrieve food from the previously unvisited closed arms. Errors were defined as entrances to arms visited previously in either the study or retention phases. Upon reaching criterion, the rats were then divided into two groups of 5 animals each. The VMT group received anodal electrolytic lesions (1 microm for 10/sec) in the VMT. In the sham group, the electrode was lowered but no current was passed. The memory test was administered once daily for 9 days. VMT lesioned animals displayed significantly more errors than did sham operated rats.

Supported by the Emory University Research Fund.


The role of the hippocampus in spatial and cued discrimination learning was examined in two experiments in a modified, cue-controlled task. (1) The effects of hippocampal lesions on performance, (2) the effects of performance on protein kinase C (PKC) in the hippocampus. The spatial discrimination (SD) and cued discrimination (CD) were equivalent in terms of the sensory and motor components, but differed in the stimuli that were relevant and irrelevant to the discrimination. Hippocampal lesions (HPC) impaired performance relative to controls (CON) in the SD, but not the CD. Mean response time (and standard error) to reach the submerged platform on the final block of trials was: SD-HPC=12.6(1.6), SD-CON=3.2(0.3), CD-HPC=5.1(1.1), CD-CON=3.2(0.3), each N=8. Group alterations in PKC were determined by autoradiography in separate groups of rats after 2 or 9 sessions of training (8 trials per session) in either discrimination.


Damage to the projection from the medial septal nucleus (MS) to the hippocampus has been shown to disrupt spatial working memory more than it disrupts nonspatial working memory (Aggleton, Hunt, & Rawlin, 1986). To further test this hypothesis, rats with MS lesions were first trained on a spatially delayed matching to sample (DNMTS) task in a Y-maze task and then on a nonspatial DNMTS task in the same Y-maze. The spatial task required the rats to enter the arm opposite that which they were forced to enter on the preceding run in order to obtain 5% of 0.2% saccharin solution. As anticipated, rats with small MS lesions were less accurate in selecting the opposite arm during acquisition when the retention interval was 10 sec. Moreover, in subsequent tests when the retention interval was varied between 5, 1, and 2 min, the deficit increased as the retention interval increased. The nonspatial task required the rats to enter the arm containing the object not encountered in the straight alley on the preceding forced run. In contrast to the spatial task, MS lesions did not disrupt accuracy of choice during acquisition or during subsequent tests when the retention interval was varied. Although it is possible that the difference in the effects of the lesions on these two tasks could be due to differences in task difficulty or to recovery of function, these possibilities seem unlikely. Thus, these results suggest that rats with small MS lesions have more difficulty remembering where they have been than they do remembering what object they have just seen.

511.6 Hippocampal Lesions in Rats Produce a Temporally-Graded Retrograde Amnesia on a Spatial Memory Task. J. J. Kubie, S. Davayani, R. U. Malinar, B. Cohen, B. Major and R. J. Sutherland, S.U.N.Y. Health Science Center, Brooklyn NY and University of Lethbridge, Alberta, CAN.

A temporally-graded retrograde amnesia is a characteristic of the human amnestic syndrome. A recent water maze study by Sutherland et al suggests a parallel in rats. Animals trained 14 weeks before hippocampal lesions were able to reacquire efficient behavior while those trained immediately before surgery were severely impaired on reacquisition (Nanouei & Ader, 1987).

In a study reported last year we attempted to further investigate this phenomenon with another spatial task—an appetitive task run in a dry, cylindrical enclosure (Kubie et al, Neurosci. Lett. 186, 1995). We found that when rats were trained 14 weeks before surgery had no retrograde loss—they rapidly reacquired the spatial task. In contrast, the same rats exhibited an anterograde amnesia in that, after hippocampal lesions, they could not learn to navigate towards a new location. The current study is an extension of our previous work and asks two questions: Is there evidence of temporally graded retrograde amnesia and, if so, is the retrograde amnesia specific to spatial memory?

In the first experiment, using procedures identical to the earlier study, rats were trained on the spatial task within a two week interval, and then immediately subjected to hippocampal (n=5) or sham (n=6) lesions. After surgery rats with hippocampal lesions were severely impaired, and 4 days required spatial abilities on the spatial task. This is in contrast to previous data where rats with a 14 week interval between training and surgery were relatively unimpaired. Thus, there is support for a temporal gradient in the retrograde amnesia.

In the second experiment, rats were trained in an object discrimination task where each rat was rewarded for digging below one out of 12 objects in an open chamber. Following a 2-week acquisition period rats were given hippocampal (n=3) or sham (n=3) lesions. After recovery, all rats showed perfect retention of the preparative habit. Thus, the retrograde amnesia is not seen in rats that are at least somewhat specific to spatial memory. (Supported by NIH grant RO1-N36096).


Medial septal lesions disrupt cholinergic input to the hippocampus and produce behavioral deficits similar to those observed following hippocampal lesions. In the current study, however, medial septal lesions differentially affected acquisition of two spatial memory tasks generally regarded as being sensitive to hippocampal damage—the radial arm maze and the Morris water maze. Radiofrequency lesions of the medial septum (MS) were made in male, Long-Evans rats. These rats and nonoperated controls were then trained in a Morris water maze to find a cannulated escape platform located in a fixed position across trials. Escape latencies for MS-lesioned and control rats were not significantly different, and analysis of search patterns during a probe trial conducted in the absence of an escape platform revealed similar results. When these rats were later trained on the radial arm maze, however, the MS-lesioned rats displayed marked impairments that persisted throughout a 15-day training experience [p<.001]. Thus, these MS lesions impaired spatial learning in the radial arm maze, but not in the water maze.

Experimental with a different set of rats revealed MS lesion-induced impairment of short, within session habituation of locomotor activity in an open field, but no effect on habituation of activity observed across days. This finding may be related to the differences observed between the effects of these lesions on the radial maze and the water maze since information regarding platform location is typically maintained over longer periods than is information regarding arms visited in the radial maze. The distinction, however, does not appear to be related to arbitrary, experimenter-imposed working memory manipulations, as further work revealed no lesion effect on the performance of a "working memory" version of the water maze in which a new platform location was used each day.
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990


This study was aimed at dissociating the role of the posterior parietal cortex (PPC) and hippocampus (HPC) in exploring and in reacting to spatial novelty. In two experiments, rats were first allowed to explore an open field containing one or several objects (habitation phase). Then a change was brought to the initial situation either by removing the object (Experiment 1) or displacing two objects among five (Experiment 2). Both changes could be detected on the basis of the spatial array only. The behavioral reaction to the change was measured by the time spent exploring.

Rats with PPC lesions or HPC lesions were tested. We also investigated the effect of reversible short-lasting inactivation of the hippocampus in rats chronically equipped with cannulas implanted into the ventral hippocampus and injected with lidocaine. By comparison with control rats who spent more time at the location of the disappeared stimulus (Experiment 1) or who reexplored selectively the displaced objects (Experiment 2), the hippocampally damaged rats did not display any reaction to change in either experiment. As hippocampal rats, animals with PPC lesions did not react to the change in any of the two experiments. In contrast, in Experiment 2, rats with PPC lesions reexplored the whole set of objects. Therefore, the role of PPC appears to be more subtle than that of HPC. PPC may have a particularly important role in discriminating spatial environmental changes that result from the absence of familiar visual cues.


The present study was aimed at testing the effects of a reversible inactivation of the hippocampus and of the septum on the learning of the radial arm maze and short-term spatial learning in the rat. In a circular platform with 18 holes on the periphery, rats chronically equipped with cannulas into the ventral hippocampus or the septum were trained to locate the one unique hole leading to a hidden shelter in order to avoid a bright light. In Exp.1, the task emphasized long-term acquisition of spatial information (1 trial/day for 16 days, 24 hrs ITI). The location of the hole visited on the 16th day and rats were injected with lidocaine just before each of the further 8 daily trials. Both lidocaine-injected and sham-injected rats learned the new location at a similar rate. In Exp.2 which emphasized short-term acquisition of spatial information (3 trials/day, 1 min ITI), only sham-injected or lidocaine-injected on alternate days and had to learn a new hole location each day. While sham-injected rats improved in orientational accuracy over successive trials, lidocaine-injected rats did not. These results confirm the role of the septum and hippocampus in spatial learning. However, rats were impaired only when septo-hippocampal activity was neutralized over the whole course of spatial processing (Exp.2). In contrast, inactivation of the septo-hippocampus during restricted periods of a longer-term process did not seem to prevent normal learning in spatial learning (Exp.1). It is hypothesized that the septo-hippocampal formation could process information "offline" in the delay between two learning trials.


Three experiments examined the effects of fenobarbital-tinntoxin on the orientation of discrete-trials delayed alternation (DA) and position discrimination (PD) in a T-maze (for procedures see Green & Stanton, Pharmacol. Rev., 1989, 41:51-105). In Experiment 1, 23-day-old rat pups given lesions on postnatal day 10 (PND10) acquired PD, but not DA after 60 training trials. Experiment 2 showed that this same lesion prevented the developmental emergence of DA between PND14 and PND27. Experiment 3 showed that 23-day-old pups with lesions did not begin to show alternations in DA if given 72 additional training trials. Lesions were verified by histological examination of the fornix and anterior thalamic nuclei in the hippocampus. The septo-hippocampal projection appears to be necessary for the orientation of DA but not PD in the rat. The lack of effect on PD suggests the lesion selectively impairs memory processes subserving DA. These findings suggest a role for septo-hippocampal maturation in the orientation of these memory processes.


Two experiments examined the effects of medial prefrontal cortex aspiration lesions on acquisition of T-maze delayed alternation (DA) and position discrimination (PD) in infant rats (see Green & Stanton, Behav. Neural Biol., 1990, 53:98-105, for experimental details). In Experiment 1, rat pups given lesions on postnatal day 10 (PND10) and trained on PND32, acquired PD, but not DA after 60 training trials. Experiment 2 showed that this lesion-induced impairment was not longer evident in animals trained on PND33. Lesions were verified by histological examination of cresyl-violet stained brain sections. The medial prefrontal cortex appears to be involved in DA but not PD during early development in the rat. The differential effect on these two tasks suggests a selective impairment of the memory processes subserving DA. The failure of this early lesion to impair DA learning in adult rats therefore appears to reflect recovery rather than sparing of function (Roth & Norenstein, Brain Res., 1978, 152, p. 149). This recovery may occur between 23 and 33 days of age in the rat.


Regional mapping of 14C-glucose (GLU) uptake was analyzed in mice at different time intervals both the first (Day 1) and last (Day 9) daily sessions of a spatial discrimination testing procedure in an eight-arm radial maze. BALB/c mice with a junctural core were rotated in each trial, and the core was divided into four groups: two experimental groups, respectively trained for 1 day and 9 days in the spatial discrimination task and two (quiet and active) control groups. Each experimental group was divided into three subgroups injected with GLU at different post-training (PT) intervals (30, 1 and 3 hr), the animals were sacrificed 5 min later and the brain sections were processed. The imaging of GLU was analyzed using the relative glucose uptake method. On Day 1, a progressive increase of labelling was found in trained animals for the medial septal and the medio-dorsal thalamic nucleus (3 min PT), the hippocampal formation (1 hr PT) and the subiculum and the frontal cortex (3 hr PT). In contrast, on Day 9, increased labelling was found 5 min PT in all the previously mentioned areas and 1 hr PT, no significant labelling was found in these structures.

These results suggest that the sequential nature of the activation observed on Day 1 represents the time-dependent progressive organization of memory.

Supported by CNRS and DRET grants.


Some of the deficits observed in the human Korsakoff syndrome (impairments of temporal order judgments, exaggerated vulnerability to interference) have been attributed to a frontal lobe damage even though the frontal cortex of these subjects is not systematically. Given the common presence of diencephalic damage in Korsakoff subjects, it remains possible that these diencephalic damage are involved in hippocampal or the septum on long-term and short-term spatial learning in the rat. In a circular platform with 18 holes on the periphery, rats chronically equipped with cannulas into the ventral hippocampus or the septum were trained to locate the one unique hole leading to a hidden shelter in order to avoid a bright light. In Exp.1, the task emphasized long-term acquisition of spatial information (1 trial/day for 16 days, 24 hrs ITI). The location of the hole visited on the 16th day and rats were injected with lidocaine just before each of the further 8 daily trials. Both lidocaine-injected and sham-injected rats learned the new location at a similar rate. In Exp.2 which emphasized short-term acquisition of spatial information (3 trials/day, 1 min ITI), only sham-injected or lidocaine-injected on alternate days and had to learn a new hole location each day. While sham-injected rats improved in orientational accuracy over successive trials, lidocaine-injected rats did not. These results confirm the role of the septum and hippocampus in spatial learning. However, rats were impaired only when septo-hippocampal activity was neutralized over the whole course of spatial processing (Exp.2). In contrast, inactivation of the septo-hippocampus during restricted periods of a longer-term process did not seem to prevent normal learning in spatial learning (Exp.1). It is hypothesized that the septo-hippocampal formation could process information "offline" in the delay between two learning trials.

In polygamous voles species males range more widely than females in the field and perform better on laboratory measures of spatial ability; both differences are absent in monogamous vole species. Such cognitive differences, predicted by theories of sexual selection, should be reflected in the size of the hippocampus, a structure known to mediate spatial learning and whose size in inbred mouse strains, correlates positively with maze performance.

Ten females and males were taken from wild populations of two vole species. The volume of the hippocampus, relative to the entire brain, was determined from serial brain sections. Males of the polygamous species, Microtus pennsylvanicus, had significantly larger hippocampi than did females, whereas there was no sex difference in hippocampal size in the monogamous species, M. penniculus. Our result suggests that the structure of functional subunits in the brain can be shaped by evolutionary processes to meet particular cognitive demands. In Microtus, the pressures of sexual selection may have produced adaptive sex differences in brain structure and behavior.


Ibotenic acid or AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate) was infused into the nucleus basalis of Meynert (nBM) of rats. Acquisition and performance of a water maze over a 10-day period (2 trials/day) were unaffected by AMPA-induced lesions which caused an 80% decrease in cortical choline acetyltransferase (ChAT) activity. However, acquisition, but not performance, was significantly impaired by ibotenate lesions, which caused a 60% reduction in cortical ChAT activity. Ibotenate-lesioned rats swam further in the training quadrant during a probe trial when the platform was removed, than either AMPA-lesioned or control rats, suggesting a perseverative search strategy. Neither ibotenic nor AMPA, lesions affected step-through passive avoidance (PA) acquisition. However, rats with AMPA, but not ibotenate, lesions were significantly impaired in the reversal test. Histological analysis revealed that AMPA infusions destroyed more nBM ChAT-immunoreactive neurons than did ibotenate infusions but, unlike ibotenate infusions, spared the overlying globus pallidus and many parvocellular neurons of the ventral pallidum. The ibotenate-induced impairment in the water maze appears to be related to pallidal, but not nBM, damage. The AMPA-lesioned impairment in PA retention appears to be more directly related to destruction of magnocellular cholinergic nBM neurons.

511.18 EFFECTS OF MEDIAL SEPTAL LESIONS ON ACTIVITY AND WATER MAZE PERFORMANCE. John J. Boitano, Carl E. J. Dobla, Sean Parker, Kristina Stalsler, Nanci Norelli*, Melissa Fiorini*. Dept. of Psychology, Fairfield University, Fairfield, CT 06430, & St. Anselm College, Manchester, NH 03102.

The effects of electrolytic lesions of the medial septal area (MSA) in F-344 rats was examined using 2 activity measures and reversal learning of the Morris water task. In the circular open field, the number of areas entered by control and lesioned A's were contrasted over 8 days (5 min/day). In a closed field situation, all A's were given forced confinement in an empty goal box (40 mins over 4 days), and were then placed in the field for 5 daily sessions. Latency to head-poke into the goal box plus the areas traversed were converted to a velocity index. Both locomotion and velocity were significantly reduced in the MSA rats (p < .01). Acquisition in the water maze over 16 days revealed insignificant group differences. In a test of reversal learning, one month later, MSA rats took significantly longer to locate the hidden platform (4 days, 2 trials/day; p < .0003). While the findings support previous research concerning hypoactivity in MSA A's, they raise unanswerable and dubious as to whether neuronal changes taking place after acquisition.

511.19 BEHAVIORAL EFFECTS OF COMBINED CHOLINERGIC AND NORDRENERGIC LESIONS. D.J. Conner, P.J. Laplaca and L.J. Thal. San Diego VAMC and Dept. of Neuroscience, UCSD, La Jolla, CA.

To examine the behavioral effects of combined lesions of the cholinergic and noradrenergic systems, we lesioned the nucleus basalis magnocellularis (nBM) and dorsal noradrenergic bundle (DNB) of adult F-344 rats (280-300g) with ibotenic acid and 6-OHDA respectively. ChAT activity was significantly decreased from controls in both the nBM and nBM/DNB groups (depletion % vs. control: nBM = 4%, nBM/DNB = 2, and 33.618 ± 6.4). NE levels were depleted by >90% by the DNB lesion with no effect of the nBM lesion of the DNB lesion. Acquisition of the Morris water maze showed an impairment of performance by the nBM lesion and an independent enhancement of performance by the DNB lesion. Reversal of the task revealed a similar pattern with the DNB lesion decreasing the behavioral effects of the nBM lesion. A water-motivated T-maze using a nonmatch-to-sample paradigm showed impaired acquisition by the task by all lesioned groups. An open field maze showed no significant differences in horizontal activity between groups. These results suggest independent effects of the two lesions, and that NE depletion is able to reverse the cholinergic deficit in some tasks.

511.20 MOUSE STRAIN DIFFERENCES IN MORRIS WATER TASK PERFORMANCE. B.F. Petrie and N.M. Standish, Department of Psychology, Red Deer College, Red Deer, Alberta, Canada, T4N 5H5.

Pigmented and Albino mice were swum for 30 days, to test for spatial learning ability, in the Morris water task. Albino animals were unable to reliably find the platform, while the time taken by Pigmented mice in escaping the pool declined rapidly, reaching asymptote within 7 days. Results are discussed in the context of genetic differences in the appropriate selection of strategy, that may have correlative anatomical and neurochemical underpinnings. Implications for memory augmentation and deficit are also analyzed.

The avian song system is an interconnected group of brain nuclei that are believed to be responsible for song production in songbirds (Nottebohm et al., 1976). The telencephalic nucleus most directly implicated in song production is HVC. There is considerable variation in the volume of HVC in males, both within and between species. This variation is correlated with syllable repertoire size in canaries (Nottebohm et al., 1981), but not in sparrows and blackbirds (Kim et al., 1983). The number of marsh wrens, HVC is larger in birds from the one with larger syllable repertoire (Canaday et al., 1984). These studies suggest that HVC volume is somehow related to song capacity, but the nature of the relationship is unclear. In the present study, the volumes of HVC, the telencephalon, and the parahippocampi were calculated for 26 species of songbird. In this sample (LH) varied in volume from 312 mm³ (chiff chaff) to 5728 mm³ (maori). Telencephalon and HVC volume are positively correlated (r = .41). Telencephalon and parahippocampi volume are more closely related (r = .88). Thus, most of the variation in the volume of the parahippocampi can be accounted for by allometric relations, most of the variation in HVC volume cannot. The residual HVC volume was then compared to indices of species-specific song duration and variety (Hamilton and Zuk, 1982; Read and Weary, 1990). Preliminary analyses suggest that the remaining variation in HVC volume is not related to song duration or the number of different syllables in a bird’s repertoire, but is related to the number of different songs that a bird is able to produce. Supported by HD 2103, Royal Society (UK), SERC (UK).

512.3 BILATERAL LMAN LESIONS IN ADULT MALE CANARIES AFFECT ACOUSTIC PERFORMANCE DIFFERENTIALLY. R. Suter, A. Tolles, M. Nottebohm and F. Nottebohm. Rockefeller Univ. Field Research Center, Millbrook, NY 12546. The lateral magnocellular nucleus of the neostriatum (LMAN) was bilaterally lesioned in 6 adult male canaries (old) males in mid-September, when their song was very variable. The song of these birds was recorded during the following periods: the birds were kept in captivity and their brains prepared for histology. Immediately after the lesion song became extremely stereotyped -- so much so that canaries in breeding condition -- successive repetitions of a same syllable being virtual carbon copies. However, within 2-3 weeks syllable diversity plummeted and birds started to produce uncommonly long tirades of just a few syllable types. These few syllable types were often delivered in a stammering fashion. We infer that in adult canaries in end-of-summer plastic song the recursive circuit to which LMAN belongs is necessary for song plasticity and for the acquisition, production and sequencing of complex learned repertoires.

512.5 NEURONAL CLUSTERS: INCORPORATION SITES FOR NEURONS IN THE ADULT CANARY FOREBRAIN. J.R. Kim and F. Nottebohm, The Rockefeller University, Field Research Center, Tyrrell Rd, Millbrook, NY 12546.

Neurons generated in the adult canary telencephalon are incorporated into vocal control nucleus HVC (Higher Vocal Center), where half or more become long-term neurons within the effector pathway controlling learned song (Alvarez-Buylla & Nottebohm Soc. Neurosci. Abstr. 383.10, 1989; Kim & Nottebohm, Soc. Neurosci. Abstr. 383.9, 1989). Some new neurons also make direct soma-soma connections to HVC, forming neuronal “clusters” (Burd & Nottebohm, ICBN, 240.143-152, 1985). In the present work, we have identified and identify neuron types found in clusters. Each of 9 adult male canaries (17 months) received a total of 14 injections of 4-hydroxy-3-didymine (6.7 Cimoleule, 2.5 ug/ml bw, 2 injections/day over 7 days). The 60-70 day labeling period was followed by uptake studies processed for autoradiography and counterstained with cresyl violet. Roughly 40% of H-labelled HVC neurons (n=36; ranges: 30-52%) were in clusters containing 2-4 neurons, which were probably an underestimation due to cell/cluster splitting during tissue sectioning. The projection patterns of clustered neurons were explored in additional male canaries (n=5) which received stereotaxic injections of the retrograde tracer Di-I or Fast Blue into Area X and Flore-gold into RA (Robustus Archistriatum), the two targets for HVC projection neurons. A common cluster arrangement of a single Area X-projecting neuron, surrounded by 2-3 RA-projecting cells was observed. Thus, the major projection pattern of HVC are related in smaller neuronal assemblies. Neuron clustering is associated with synchronized activity in other systems (Theodosius et al., Nature 332: 738-40, 1986). In HVC, the activity of newly formed neurons may be constrained by that of preexisting neurons within clusters—a potential mechanism for the preservation and amplification of information in a system which undergoes turnover of some of its elements. It is also possible that newly formed neurons alter cluster responses properties.


Six adult male canaries were deafened, 7 served as controls; 2 weeks later both groups got 6-7 implants and were placed facing cages with singing male. All females received injections of 3H-thyminide (50ugI per inj., at 12h intervals) starting three days later. Their brains were processed for autoradiography, and six weeks after the last injection. The boundaries of the high vocal center (HVC) were defined using cresyl violet. The HVC of deaf and hearing birds did not differ in volume, number of neurons, glia and endothelial cells, area of overlying ventricular zone (V2) and % of labeled neurons. However, the % of labeled V2 cells overlying HVC and the % of labeled HVC glia and endothelial cells were higher in the deaf than in the hearing birds (RLS side combined). Interestingly, the effect on glia and endothelial cells was significant only on the right side. Mean nuclear diameters of labeled HVC neurons were larger on the left than on the right side of hearing birds. Deafness affected turnover and differentiation of HVC cells, particularly on the right side.

512.6 EFFECT OF UNILATERAL DENERVATION ON THE ACOUSTIC OUTPUT FROM EACH SIDE OF THE SYRINX IN SINGING MIMIC THRUSHES. R. A. Suthers and R. S. Harris*. Sch. of Med. and Dept. of Biology, Indiana Univ., Bloomington, IN 47405.

Microbead thermistor implants in each primary bronchus of adult male carbirds (Dumetella carolinensis) and brown thrashers (Geothlypis trichas) were used to record the acoustic contribution of the syrinx before and after unilateral denervation of the syrinx muscles. Thermistor responses to air movement through the vibrating syringal membranes. In these birds, paralysis of the muscles on either side of the syrinx resulted in an abnormally loud, but the effect was usually slightly greater after the left side was paralyzed. Denervation of either the left or right side of the syrinx increased the number of syllables to which side contributed sound. Few or no syllables were produced by the intact side and most post-denervation thermistor responses contained simultaneous contributions from both sides of the syrinx. The sound generated on the denervated side usually consisted of a fundamental with an abnormally low frequency and multiple harmonics. The frequency of this fundamental typically paralleled changes in the rate of airflow on that side of the syrinx, which in turn followed changes in the driving sub syringal pressure. Sound from the intact side was essentially normal. The abnormal sound in these birds is primarily due to their inability to regulate resistance or membrane tension on the operated side of the syrinx. We postulate that since the denervated side can no longer act as a mechanical dead space, a more direct medialis tympaniform membrane vibrates at a frequency determined by the rate of airflow across it. (Supported by grant BNS 87-20192 from N.S.F.).
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

1520 THURSDAY PM

512.7 GABAERGIC INHIBITION CONTRIBUTES TO NON-LINEAR SUMMATION AMONG MULTIPLE FREQUENCY CHANNELS IN THE BARN OWL'S INFERIOR COLICULUS. K. Morita*, I. Fujita*, S. Komatsu, 1Giv. of Biology, Pa. State University, University Park, PA 16802 and 2Lab. for Neuroinformation Processing, RIKEN, Wako, Saitama 351-01 Japan.

Interaural time difference (ITD) is the primary aural sound localization cue for the barn owl. Since ITD is processed in separate frequency channels in the brainstem pathways, neurons therein clearly distinguish ITD from interaural phase difference. Instead, they respond to multiple ITDs that give rise to the same phase difference (phase ambiguity). However, in the external cells and some cells in the lateral shell of the central nucleus (IC, type 2 cells of Fig.1, Area), we observed in the avian species. In the external cells and some cells in the lateral shell of the central nucleus (IC, type 2 cells of Fig.1, Area), we observed in the avian species. In the external cells and some cells in the lateral shell of the central nucleus (IC, type 2 cells of Fig.1, Area), we observed in the avian species. In the external cells and some cells in the lateral shell of the central nucleus (IC, type 2 cells of Fig.1, Area), we observed in the avian species.

512.8 Focusing in Owls and Raptors Measured by Photoresponse. H. Howland, M. Howland & J. D. Pettigrew, Division of Biological Sciences, Cornell University, Ithaca, NY, I4855 & Vision Touch and Hearing Research Centre, Department of Psychology, University of Queensland, St. Lucia, Qld. 4067

Previous work on a number of species of owls has shown that these birds exhibit a great variety of accommodation abilities which are roughly correlated with their size. Large owls exhibit small accommodative ranges while small owls generally exhibit large ones. An exception to this rule is the (medium sized) barn owl, Tyto alba, which showed a greater than 10 diopter accommodative range (1). We have initiated an investigation of the focusing abilities of Australian owls and raptors including three species of tyto owls, the boobook, Ninox novaeseelandiae, and a species of the night owl, Strix aluco. We have employed both orthogonal photorefrerence and photo-retronscopy, using for the latter both infra red and visible light. We have found that the speed and range of accommodation of the owls is roughly that of the Australian Tytonidae. We obtained a clear neutralizing photorefractive refraction of the sparrowhawk (Accipiter cirro- capillatus) showing that it accommodates through 10 dioptries. Particularly interesting was the fact that the peripheral fields of the hobby falcon (Falco longipennis) and the sparrow hawk exhibited considerable heteroesthesia. The implications of the focusing abilities of these birds and the optical qualities of their eyes for their visual capacities will be discussed. Facilities permitting, several of the optical refractive techniques will be demonstrated. Supported in part by NSF BNS-890994 & a Fogarty fellowship to HCH and a grant from the ARC to JDP.


512.9 ACUOSTICALLY RESPONSIVE UNITS IN THALAMIC REGIONS AFFECTED TO THE AVIAN BASAL GANGLIA. S.E. Dugan*, R.M. Tepper & M.F. Chong, Institute of Animal Behavior and Center for Molecular and Behavioral Neuroscience, Rutgers University, New Brunswick, New Jersey 08901

Recently, Area X a region within the optic parafacial lobe (PO) that is a component of visual pathways, was investigated in the pigeon. In the ring dove (Streptopelia risoria), a non-vocal leaser, caudal lateral PO has not been demonstrated to exhibit a specialized subdivision associated with vocal pathways. However, in the dove, caudal PO apparently has a somatotopy from the thalamus (PO). In the pigeon, a thalamic region, caudal to the thalamic projection of Area X in songbirds, contains acoustically responsive units and is retrogradely labeled from caudal PO. The possibility that caudal PO is a region of the basal ganglia involved in the control of motor integration, with a unique role to the thalamus, is a question deserving further investigation across avian species.

The projection from Ov to caudal PO and the medial pallium/nucleus from Area X and PO to the nucleus have been demonstrated to exhibit a specialized subdivision associated with vocal pathways. However, in the dove, caudal PO apparently has a somatotopy from the thalamus (PO). In the pigeon, a thalamic region, caudal to the thalamic projection of Area X in songbirds, contains acoustically responsive units and is retrogradely labeled from caudal PO. The possibility that caudal PO is a region of the basal ganglia involved in the control of motor integration, with a unique role to the thalamus, is a question deserving further investigation across avian species.

During our study of the acoustically responsive neurons of Area X, four acoustically responsive units in the DM/PD border region were recorded in urethane anesthetized animals. In the absence of acoustic stimulation, these units exhibited population responses, ranging 0.2 to 0.5 spikes and having long latency responses (LSR) of 250-500 ms relative to the time-locked burst. Tone pures of 200 Hz elicited bursts of activity from 2 of these units; one also responded to 500 Hz tones. A more dorally located unit only showed weak responses to frequencies in the range of 2 kHz. A minor projection from Ov to caudal PO was revealed previously by Ivers L, etc., but it may not constitute the source of auditory input to these units. Supported by Johnston and Johnston patterning in brain and by the Japanese Ministry of Education, Science and Culture.


The role of forebrain visual structures in the sensorimotor control of eating in the pigeon was examined using a technique combining unilateral hemispherectomy with monocular occlusion to produce a reversible "visual deafferentation". Given the hodology of the visual system (complete decussation of the visual pathways to the forebrain) input to the eye contralateral to the intact hemisphere will access, unilaterally, all components of the tectofugal and thalamofugal pathways. For the eye contralateral to the ablated hemisphere, the forebrain components of these pathways will be eliminated. By alternatingly occluding the eye contralateral to the intact hemisphere, subjects could be tested first as visually "normal" and then as visually "deafferentated".

When tested in the Binocular condition or using the eye contralateral to the intact hemisphere, subjects showed no impairment on measures of ingestive behavior, discrimination of food items, food source localization and the amplitude scaling of gape (interbreak distance) during grasping. When using the eye contralateral to the ablated hemisphere, there were significant impairments in ingestive efficiency, peck localization and the amplitude scaling of gape (interbreak distance) during grasping. When using the eye contralateral to the ablated hemisphere, there were significant impairments in ingestive efficiency, peck localization and the amplitude scaling of gape (interbreak distance) during grasping. When using the eye contralateral to the ablated hemisphere, there were significant impairments in ingestive efficiency, peck localization and the amplitude scaling of gape (interbreak distance) during grasping. When using the eye contralateral to the ablated hemisphere, there were significant impairments in ingestive efficiency, peck localization and the amplitude scaling of gape (interbreak distance) during grasping. When using the eye contralateral to the ablated hemisphere, there were significant impairments in ingestive efficiency, peck localization and the amplitude scaling of gape (interbreak distance) during grasping.

Support: NSF Grant BNS 88-10722 and NIMH Grant MH-80366.

512.10 HIPPOCAMAL LESIONS IMPAIR NAVIGATIONAL MAP DEVELOPMENT IN HOMING PIGEONS. V. P. Bingman, E. Jacob*; G. Casini* and F. Bagnoli, Dept. Psychology, Bowling Green State Univ., Bowling Green, OH 43403.

The homing pigeon navigational map allows birds to determine their position and take up an approximate homeward bearing from distant, unfamiliar locations. The navigational map is a highly specialized map with many characteristics of the "cognitive map" found in the psychological literature, and may be the best example of a naturally occurring cognitive mapping system. The hippocampus has been shown to play a critical role for spatial navigation in laboratory rodents. Surprisingly, previous research has shown that hippocampal ablation has no discernable effect on the functioning of the pigeon navigational map in adult birds with homing experience. In contrast, the present study demonstrates that hippocampal ablation in young pigeons who have yet to acquire a navigational map strikingly impairs their latter ability to take up a homeward bearing from distant unfamiliar locations. The data suggest that the avian hippocampus plays a critical role in navigational map acquisition while playing no further necessary role once the map is formed. Alternative explanations will be explored.

512.12 AUTODRAGOGRAPHIC LOCALIZATION OF MUSCARINIC CHOLINERGIC RECEPTORS IN THE BULBIFORM BRAIN. P. F. Balf, Dept. of Psychology, Boston College, Chestnut Hill, MA 02167

Bulbiform (tectotectal/uniocular) nucleus is a parietal member of the parabelt, possess a neural vocal control system that is possibly homologous, at least in part, to the complex nuclei that control vocal behavior in the songbird auditory system. A comparison of the transmitters present in the song control nucleus in these two distantly related avian groups may address the question of homology and clarify whether vocal behavior is regulated in a similar fashion in these two taxa. I therefore used in vitro autoradiographic autoradiography to map the distribution of muscarinic cholinerigic receptors, a receptor known to be present in several song control nuclei in songbirds, in male and female bulbiform nuclei. Muscarinic receptors were labeled using [3H]Methylscopolamine (1 nM conc. ± 5% [methyl] atropine. Sites were exposed to tritium sensitive film for 1 week. Autoradiograms were analyzed using an imaging system at the level of the septum, habenular nucleus. The song control nucleus, the nucleus intercollicularis (IC) is in the mid-brain was specifically labeled. No structure could be discerned that resembled the robust nucleus of the archistriatum. At the level of the anterior commissure a lateral structure exhibiting high specific binding was detected that was near the putative homologue of the caudal part of the ventral tegmental nuclei. Dorsally at this level, it did not appear to correspond precisely to this nucleus. These data suggest potential differences in the regulation of vocal behavior in parrots and songbirds.
131.1 EFFECT OF DIETARY FAT SOURCE ON MACRONUTRIENT SELECTION
B. J. Mullin and R. J. Martin, Dept. of Foods and Nutrition, Dawson Hall, Univ. of Georgia, Athens, GA 30602.
We have demonstrated that type and level of dietary fat can influence an animal’s subsequent preference for carbohydrate (CHO) and protein (PRO). Rats fed a 34% tallow diet show a subsequent preference for a high PRO diet while those fed 34% corn oil show a preference for a high CHO diet. The present study was undertaken to determine if the factor(s) responsible for restricting PRO consumption in the tallow fed animals is restricted adipose tissue. Hence, we tested the effect of another beef fat, butter, which is derived from secretion rather than a byproduct of adipose tissue. Male, Sprague-Dawley rats (75-99 g) were divided into 3 groups and fed diets containing either 34% corn oil, tallow or butter for 2 days. These diets were then replaced with 2 diets given simultaneously to test dietary selection: 1) 15% CHO/60% PRO and 2) 65% CHO/10% PRO. Animals previously fed corn oil selected more CHO and less PRO than did animals fed either tallow or butter. The amounts of PRO and CHO selected by animals fed tallow or butter were not significantly different. These results suggest that the factor(s) responsible for altering diet selection are present in both adipose tissue and milk fat.

Of the numerous metabolic functions of corticosterone (Cort), virtually all can be assigned to the monone de- creased from the type I receptor. This essay was written to test as the role of the high density/low affinity type II receptor. The endogenous type II signal is relatively brief and is delivered against a background of type I saturation. The present study examined the relative effectiveness of this pattern of signalization. The type II agonists, dexamethasone (DEX) and RU 28362 (RU) were chronically administered in the presence or absence of continu- ous type I stimulation. DEX and RU were delivered by continuous infusion or by single daily injections at 7 pm. Administered alone, RU and DEX reduced body weight gain, feeding efficiency, and food intake. Continuous infusion was considerably more suppressive than injection and pro- duced wider fluctuations in daily body weight gain. The suppressive effects of DEX and (especially) RU were at- tenuated when type I receptors were occupied; the lowest dose of RU (5ug/day) slightly augmented the strong anabolic effect of the type I agonist (Aldo). The outcome of type II receptor stimulation remained dependent on mode of administra- tion and concurrent type I activity. Supported by DK 34347 to L.D.

131.5 RATS OVERFED AS NEONATES BECOME OBESE AND INSULIN RESISTANT AS ADULTS. J. A. C. McGarvey, G. J. Watts, and A. Scheuer, Dept. of Psychology, University of Washington, Seattle WA 98195.
Animal models of the various aspects of non-insulin dependent diabetes (NIDDM) have typically used mutant rat strains and or severe pharmacological insults such as streptozotocin lesions of the pancreas. This experiment precipitated aspects of NIDDM with environmental manipulations during development. At four days of age, pups were randomly assigned to one of three groups 1) mother reared in litter of ten (MR) 2) gastrostomy-fed for nine days (Days 5-14) to match the growth of the MR group 3) MR fed with the formula designed to accelerate growth (OF). At Day 14 the gastrostomy-fed groups were returned to dams. All animals were weaned on Day 21. Females were paired with males on Day 55. At 6-7 weeks of age a successful pregnancy received IV glucose challenges via chronic jugular catheters. Overfeeding became frankly obese after puberty. Only the less severely obese females were able to carry a pregnancy to term and their pups were significantly smaller than the offspring of feeding controls. An exaggerated insulin response to the IV glucose challenge was seen.

These data demonstrate that nutritional manipulations early in life may produce an adult with some of the characteristics of NIDDM.

131.2 ABSTRACTS AND CORRECTED REPLACEMENTS: EFFECTS OF MACRONUTRIENT INTAKE. P. DiPietro, R. Yamamoto, T. Eker, and S. L. Itagaki, Rockefeller Univ. N.Y. 10011. Adrenalec- tomy (ADX) decreases food intake and body weight and prevents the development of various forms of obesity. We have examined macronutrient intake patterns in rats with complete ADX (60% blood corticosterone (CORT) levels < 2 pg/ml), "incomplete" ADX (blood CORT levels 1 pg/ml), and after low vs. high doses of CORT replacement.
Results indicate that, in complete ADX rats, both 24hr carbohydrate intake (<8%; p<0.05) and 24hr fat intake (>8%; p<0.05) are decreased, whereas in incomplete ADX rats only fat intake is suppressed (<4%; p<0.05). This suggests that low circulating CORT levels are sufficient to maintain 24hr carbohydrate but not fat intake. This suppression of carbohydrate feeding occurs almost exclusively during the first 2 hrs of FIV injection. This may be due to the presence of catabolites and/or increased fat intake. In contrast, the decrease in fat intake is only a small portion (20%) of the total 24hr fat suppression seen in both complete and incomplete ADX rats, which appears to occur differently throughout the feeding cycle.
Consistent with the finding that carbohydrate feeding in the early dark is lost only in complete ADX rats with 12 pg/ml CORT, tests with CORT replacement show that low levels of CORT (0.05-0.5 uAg/ml, resulting in blood levels similar to CRH) do not alter natural carbohydrate feeding. These results indicate that nutritional intake is differentially affected by ADX at different times of the feeding cycle and that low levels of CORT are sufficient to maintain or restore carbohydrate intake while reducing fat intake. It is suggested that different steroid receptors in the brain may underlie these effects.

ADX gold thiglucose-treated mice reportedly increase food intake and body weight after a single IVT injection of CORT. To study the role of CORT on weight gain, Long-Evans rats were either allowed free access to chow (ADL group) or food restricted (RES) to half baseline daily food intake (98 g/day) for two weeks prior to IVT injection or sham operation. The next day rats received a single IVT injection of 100ug CORT in 2 ul propylene glycol vehicle, or 2 ul vehicle (VEH). This dose of CORT (250ug) did not influence food intake or body weight, but when administered subcutaneously, both ADL and RES groups, ADX reduced or decreased weight gain over the six days following the injection (ADL: mean doses of 1.85; RES: mean doses of 1). IVT CORT vs. VEH. ADX vs. 14.9g, for SHAM: RES: EX. ADX gained 46.6g, SHAM 74.3g). In the ADL group, IVT CORT non-sufficiently significantly increased body weight gain in ADX rats (CORT 59.7 ± 5.5g, VEH: 36.7±5.1g). It is concluded that centrally- acting CORT has a role in determining body weight gain after food restriction in non-obese rats.

131.6 EFFECTS OF LESIONING THE AMYGDALA, PARABRACHIAL NUCLEUS AND THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS ON LIPOPOVIC AND GLUCOPROVIC FEEDING. N.Y. Callegaris, B.W. Hutton* and S. Ritter, Department of VCAPP, Washington State University, Pullman, WA 99164-6520.
Previous results have shown that feeding, stimulated by mepacrine or acetylcholine injected into the lateral ventricles, is dependent on vagal sensory neurons originating in the abdominal viscera. Feeding elicited by 2-deoxy-D-glucose injection and blockade of glucose utilization (glucoprivic feeding) does not require peripheral vagal innervation, but is abolished by a lesion of the lateral hypothalamic and parabrachial nuclei. It has been suggested that the lateral hypothalamic and parabrachial nuclei are involved in the glucose sensate area (AP/NTS) lesion. In this study, lesions were placed in the central nucleus of the amygdala, parabrachial nucleus and the hypothalamic paraventricular nucleus, in known to receive visceral sensory projections from the AP/NTS region, in order to identify central neural substrates for these controls of feeding. After recovery from surgery, rats were placed on a medium fat diet for 24 days, and then tested for lipoprivic and glucoprivic feeding. Electrolyte lesions which included the central nucleus of the amygdala abolished lipoprivic, but not glucoprivic, feeding. Kainic acid lesions of the lateral parabrachial area did not impair either control. Relatively large hypothalamic lesions destroying the paraventricular nucleus disrupted both controls. Thus, areas containing projections from the AP/NTS region may be important for glucoprivic and lipoprivic feeding. The projections critical for each control may be distinct, although common neural circuits. Supported by NIH grant #DK 40498.
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

S13.7
INTEGRATIVE BEHAVIOR: BODY WEIGHT AND RATING
THURSDAY FW


High plasma levels of monosodium glutamate (MSG, s.c.) cause a selective loss of glutamate-sensitive neurons (GSN) in the area postrema (AP). The neurochemical profile of most GSN in AP are tyrosine hydroxylase (TH)-immunoreactive (IR) and/or SHT-IR. Only sub fractions of the total TH-IR and SHT-IR AP cell populations are GSN. Remaining populations are glutamate-insensitive, even with a subsequent MSG treatment. GSN were identified in the glupic feeding response subsequent to 2-Deoxyglucose (2-DG) administration. We tested whether the GSN of AP are critical to this response. We treated 120 g male SD rats with 6 mg/kg s.c. MSG. Five days later, 4 of the rats were given a glucoprivic challenge. In the 3rd hr of the 12 h photoperiod, 400 mg/kg 2-DG or saline was administered ip. in a randomized block design (N=4 each group). Intake of ground chow was computer-monitored at resolutions of 0.5 Hz and 0.1 g. A second experiment was performed using an MSG dose of 9 mg/kg. Cumulative food intake values for the 3rd post-injection period were subjected to 1-way ANOVA. In both MSG treatments, ablation of the GSN in AP failed to inhibit the glucoprivic feeding response to 2-DG (P < 0.08). We conclude the GSN of the AP are not involved in the glucoprivic feeding response. Support: HULI 37705.

S13.8
THE EFFECT OF INTRAVENOUS INSULIN ON CEREBROSPINAL FLUID (CSF) BIOGENIC AMINE CONCENTRATIONS IN ANESTHETIZED RATS. Thomas W. Castonguay and Wayne J. Kuehn. University of Maryland, College Park, Maryland 20742

Intact rats will self-administer insulin i.v. if the concentration of substances, in serum or dopamine metabolite concentrations in CSF. Adult rats were anesthetized, fitted with jugular vein cannulae, and injected with 1.2 IU of insulin: 100, 200 or 300 mU. CSF was collected at 0, 10 and 30 min. after injection. Analysis of CSF samples was performed using HPLC with electrochemical detection. Within 30 minutes 5-HIAA increased 13, 12 and 22 percent over baseline levels in the 100, 200 and 300 mU groups respectively. CSF DOPAC increased 50% in response to 100 mU insulin within 10 minutes of injection. Higher insulin concentrations resulted in an inverse relationship with DOPAC, with a 22% decrease observed at 30 min. These results suggest that insulin is behavioral serotonin and dopamine metabolism. (Supported in part by a grant from the Whitehall Foundation).

S13.9
HYPERINSULINEMIA AND REDUCED SODIUM EXCRETION IN DIETARY OBSESE, WEIGHT-CYCLED RATS. B.R. Contreras, Department of Psychology, University of Alabama, Birmingham, AL 35294.

The aims of the present study were to: (1) replicate the findings of Embsberger and Nelson (Am. J. Physiol., 254: R47-R55, 1988) reporting the development of mild hyperinsulinemia in diet obese Sprague-Dawley (SD) rats exposed to 4 cycles of food restriction-refeeding (weight cycling) on a sweet milk diet; and (2) determine whether the mild hyperinsulinemia was associated with changes in sodium excretion, hyperinsulinemia, pressor responsiveness to angiotensin II, and heart rate response to pharmacological blockade of the autonomic nervous system. Twenty-five male SD rats were divided among 3 groups: 1 group was given ad libitum pelleted chow (Pele); 2 groups were fed pelleted chow plus sweetened condensed milk (Misc); and 9 rats were exposed to 4 cycles of a 4-d fast alternating with 2-d of refedding pelleted chow and sweetened condensed milk (Cycled). Dietary obesity and weight cycling resulted in significant elevations in body weight and terminal fat pad and white fat pad weights, hyperinsulinemia, and reduced sodium excretion. In contrast to Embsberger & Nelson, weight cycling superimposed on dietary obesity did not alter (1) blood pressure, or (2) heart responses to sympathetic stimulation with metoprool and parasympathetic blockade with methylscopolamine. The pressor responses to intravenous administration of angiotensin were also unaffected by obesity and weight cycling. The dietary obese, weight-cycled SD rat may not be a good animal model for human obesity-related hyperinsulinemia. Supported by NIH Grant HL 38630.

S13.10
WHEN MAINTAINED ON A RESTRICTED DIET, RATS REARED IN ENRICHED ENVIRONMENTS DEFEND BODY WEIGHT BETTER THAN RATS REARED IN ISOLATION. S.C. Fowler, J.M. Chase, W.J. Kallman, and R. Hopkins*. Deps. of Psychol. and Pharm., Univ. of Mississippi, University, MS 38677.

Between the ages of 22 and 82 days rats were reared, under ad libitum food and water conditions, in either enriched environments (n=37) or in individual isolation cages (n=32). Then all rats were reared to daily 1 hr feeding for the next 78 days. At this time enriched rats were significantly heavier than the isolates, t(67)=3.275, p<0.002. In addition, the difference between pre- and post-feeding body weights significantly favored the enriched rats by 4.693, t(67)=2.822, p=0.006. These results suggest that superior defense of body weight by the enriched rats is mediated via the enriched rats eating at a higher rate than the isolates. A second replication of the first and found that (1) isolates are heavier than enriched rats when faced ad libitum and (2) limiting feeding to 1 hr/day does not abolish the brain-weight-increasing effects of enriched environment rearing. Supported by DA 05310.

S13.11

Previous studies (Levin & Sullivan, 1989) showed that rats prone to develop DIO had defective activation of autonomic brain areas to food-related cues. Here, 24 adult male SD rats were fed an HED for 3mo; half became DIO, gaining 50% more weight, while the group of rats were diet resistant (DR), with the same weight gain as 12 chow-fed rats. Fasting rats were then trained to drink lentil and glucose in 15 s and associated with a tone cue. LCGU was then assessed in 20 brain areas, using [14C] 2-deoxyglucose (Sokoloff et al., 1977). Both groups were tested with tone plus 0.15% saccharin in place of glucose. DR but not DIO rats increased LCGU 14% to saccharin intake in the n. of amygdalar plus, while overall HED rats were 14% lower in the central amygdalar n. of DIO rats. HED intake led to reduced inferior olive LCGU to saccharin and a 15% decrease in medial amygdalar n. LCGU in DR and DIO vs. chow-fed rats. Thus, both DIO and HED intake adversely affect brain autonomic areas to food related cues. This may play a role in development and perpetuation of DIO.

S13.12
Estradiol Increases Sympathetic Nervous System Activity in Retroperitoneal Adipose Tissue. S.L. Lazzarini and G.N. Wade. Neuroscience and Behavior Program and Dept. of Psychology, Univ. of Massachusetts, Amherst MA 01003.

Estradiol has both central and peripheral effects on body weight in rats. Ovariectomy (OVX) results in body weight gain (primarily by increasing fat stores) and estradiol replacement reverses these effects. The sympathetic nerves play a role in estradiol-induced fat weight losses in OVX rats. Denervation of retroperitoneal adipose tissue (WAT) attenuates fat weight loss. This effect is not altered by increasing sympathetic activity by the administration of peripheral NE. Estradiol was OVX and treated with estradiol benzoate (EB) or sesame oil vehicle. NE turnover was assessed by measuring the decline of tissue NE over time after injection of [13-3H]norepinephrine (NE), an index of sympathetic activity. NE turnover was assessed by measuring the decline of tissue NE over time after injection of [13-3H]norepinephrine (NE), an index of sympathetic activity. Estradiol resulted in a significant increase in NE turnover in OVX rats. Estradiol benzoate treatments increased NE turnover above that of EB treated OVX rats. EB or sesame oil vehicle treatment did not alter turnover.

Supported by NS 01873, DK 32936, and MH 00032.

It has been proposed that the novel anorectic agent, (D)-chlorocitric acid (CA), decreases food intake through a reduction in gastric emptying (Pharmacol. Rev., Behav., 1981). If so, CA should not decrease sham feeding, a preparation where postingestive cues such as gastric distension are greatly minimized.

Here we examine the effect of CA in rats sham feeding 20% sucrose relative to the appropriate "real" feeding condition.

RESULTS: Values are mean ± SEM % of baseline intake

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Sham Feeding</th>
<th>Per Cent Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.35 mg/kg</td>
<td>0.40</td>
<td>120</td>
</tr>
<tr>
<td>0.40</td>
<td>94.1</td>
<td>81.3</td>
</tr>
<tr>
<td>0.50</td>
<td>94.1</td>
<td>81.3</td>
</tr>
<tr>
<td>0.55</td>
<td>94.1</td>
<td>81.3</td>
</tr>
<tr>
<td>0.60</td>
<td>94.1</td>
<td>81.3</td>
</tr>
<tr>
<td>0.65</td>
<td>94.1</td>
<td>81.3</td>
</tr>
<tr>
<td>0.70</td>
<td>94.1</td>
<td>81.3</td>
</tr>
<tr>
<td>0.75</td>
<td>94.1</td>
<td>81.3</td>
</tr>
<tr>
<td>0.80</td>
<td>94.1</td>
<td>81.3</td>
</tr>
<tr>
<td>0.85</td>
<td>94.1</td>
<td>81.3</td>
</tr>
<tr>
<td>0.90</td>
<td>94.1</td>
<td>81.3</td>
</tr>
<tr>
<td>0.95</td>
<td>94.1</td>
<td>81.3</td>
</tr>
<tr>
<td>1.00</td>
<td>94.1</td>
<td>81.3</td>
</tr>
</tbody>
</table>

Tests were conducted in the a.m. after a 17 hr fast. N=8, doses are mg/kg, p.o. given 30 min before sucrose. * p < .05.

CONCLUSIONS: Since CA decreased sham feeding, its anorectic effect cannot be solely attributed to inhibition of gastric emptying. However, since CA was more potent in the real feeding condition relative to sham feeding and the time course of the response in the two feeding conditions was different, this suggests that part of CA's anorectic effect does depend on postingestive cues.


Rapid duodenal glucose infusion (3 ml/min) increased chow intake in free-fed and meal-fed rabbits. Slow duodenal glucose infusion (1 ml/min) increased food intake in meal-fed rabbits but decreased food intake in free-fed rabbits, suggesting that daily patterns of meal-taking can determine whether glucose produces satiety or hunger.

To define further conditions under which glucose produces satiety or hunger, blood glucose and insulin were measured in free-fed rabbits. After fast duodenal glucose infusion, insulin increased sharply, followed by a precipitous decline in blood glucose. Slow duodenal glucose infusion increased insulin moderately, which failed to decrease glycemic levels significantly. Tests are being conducted to determine whether feeding responses vary across trials in free-fed rabbits (as found in meal-fed rabbits) and, if so, if changes in food intake are related to blood glucose and insulin responses.

Supported by NSF grant BNS-8709982 and NIDDK grant BO 1K04 DK01897-01 to PJG.

513.15 CONDITIONED SATIETY DEPENDS ON GASTRIC AND POSTGASTRIC SIGNALS. J. Lissner*, J.P. Smith, J.D. Davis, and P. Dietsch*. New York Hospital-Cornell Medical Center, White Plains, NY 10605 and University of Illinois, Chicago, IL 60680.

When rats are given successive sham feeding (SF) tests, intake increases progressively to a maximum in 4-5 tests. Interspersing 2 real feeding (RF) tests between SF tests prevents this progressive increase in intake (Davis & Smith, 1990). This result suggests that a learned association between the flavor of the test diet and its postingestional consequences under real feeding conditions inhibits sham intake (conditioned satiety). To determine if the postingestional factor is gastric and/or postgastric origin, 10 rats equipped with a surgically implanted, inflatable, pyloric cuff and a gastric fistula were tested with a 0.8% sucrose diet on 6 cycles of 2 RF tests followed by 1 SF test. On all tests the pyloric cuff was inflated to prevent postgastric stimulation by the ingested fluid. Under these conditions sham intake increased with experience (p<.01), but not as much as when no RF tests intervened. Since no progressive increase in sham or real intake occurred when food emptied normally from the stomach during real feeding, we conclude that postgastropical stimulation is a necessary component of postingestional stimulation for normal conditioned satiety. The results also reveal a significant affect of gastric stimulation because the increase in sham intake observed when the infected food accumulated in the cuff-closed stomach during RF tests was significantly less than the progressive increase in sham intake when sequential SF tests were administered without interspersing of RF tests.

Supported by NIH MH 15455 and NIH RSA MH00149.


Food consumption drops by about 30% when running wheels are made available (Looy & Eikelboom, Physiol Behav., 45:405, 1989). This study recorded only total daily food consumption and running. The nature of the change in feeding needs to be determined.

In the present study 8 male Sprague-Dawley rats (310-325g) were housed in individual cages (as weight controls n=4) or in special cages with pellet dispensers (45mg Rodent Chow Bio-Serv) providing ad lib food. For 9 days food and water consumption patterns were recorded in 5 sec bins. Then wheels were made available for 30 days and all three behaviors recorded. Wheels were closed and food and water consumption recorded for a further 30 days.

When rats had wheel access food consumption dropped by about 40%. By 30 days it had returned to about 80% of baseline. These changes were due to changes in meal number (from 10.7 to 6.8 meals a night when the wheel was opened to 13.6 after 30 days). There was no simultaneous change in the meal size. (Work sponsored by NSERC)
514.1 GABA AND BENZODIAZEPINE BINDING IN ADULT AND PERINATAL ROENTGEN SUPRACHIASMATIC NUCLEUS. M. Lu and J. L. Fischer. Univ. of North Texas, Dept. of Biological Sciences, Denton, TX 76203.

GABA and benzodiazepine binding sites have been implicated in the control of rodent circadian rhythms. Certain GABA and benzodiazepine agonists or antagonists can produce or block phase shifts, or alter the free-running period. Interest in these systems led us to examine the effects of muscimol (GABA) and [3H]flunitrazepam (benzodiazepine) binding in rat and hamster suprachiasmatic nuclei (SCN), the primary circadian pacemaker. Receptor autoradiography was used to test for the presence of rhythms which might underlie the phase-dependency of the pharmacological effects. No diurnal differences in ligand binding were found, using 6 rats at each of 8 time points in LD 12:12. In addition, there were no differences in light versus dark at night.

514.2 ALLOXAZINE BLOCKS THE HYPNOTIC EFFECT OF TRIAZOLAM IN RATS. S.D.O'Connor and M. Radulović, Dept. of Pharmacology, Univ. of IL, Chicago, IL 60612.

Triazolam, a benzodiazepine (BDZ) hypnotic, appears to mediate several of its effects through a receptor coupled to the GABAergic system. It has been hypothesized that central effects of BDZs are mediated in part through the adenosine (ADO) system (Philibin and O'Regan, TIPS 9: 5, 1988). Recent work from our lab has demonstrated the interaction between these two systems, since chronic i.c.v. administration of a specific ADO transport inhibitor, sulforzamide, mimicked the effect of chronic administration of triazolam, by decreasing radioligand binding to ADO 2A receptor in the striatum. To further examine the role of ADO in the hypnotic effect of BDZs, we measured the effect of acute administration of an ADO 2A receptor antagonist, alloxazine (ALX) on sleep induced by triazolam. Sprague-Dawley rats given 0.1 mg/kg triazolam ip showed decreased waking (25%) and increased total sleep (10%) as compared to controls during a 6 hour recording period (p<0.05). Pre-treatment of rats with 5mg/kg ALX ip, which had no effect on waking by itself, resulted in a return to control levels of sleep when given in combination with triazolam. These results indicate that at low doses, sleep induced by triazolam may be a function of increased ADO activity which can be blocked by ADO 2A receptor antagonists.


Treatment of golden hamsters with the short-acting benzodiazepine, triazolam, is associated with acute increases in activity and phase-dependent shifts in the circadian rhythm of locomotor activity which can be prevented by prior treatment with the benzodiazepine antagonist, RO-15-1788. Since transfer to a new cage with a running wheel a few hours before the onset of activity also induces phase advances in the activity rhythm in hamsters normally housed without wheels, we conducted this study to examine the effects of administration of RO-15-1788 on the phase-shifting effect of this transfer. Hamsters housed without running wheels in constant light were treated 4 hours before activity onset with (1) transfer to a new cage with a wheel for 1 hour (RO+Wh), (2) 5 mg RO-15-1788 and transfer to a new cage with a wheel for 1 hour, or (3) 5 mg RO-15-1788 alone (RO). Phase advances induced by RO+Wh (E=6±22 min.) were smaller than those induced by Wh (E=13±24 min.) and larger than those induced by RO (E=15±28 min.), but were not significantly different from each other. The number of wheel revolutions in group RO+Wh was significantly less than those in group Wh (p<0.01). Also, the increase in total locomotor activity during the hour of treatment for group RO+Wh was significantly less than the activity increase for group Wh (p<0.05), and significantly greater than the activity increase for group RO (p<0.01). These results suggest that treatment with RO-15-1788 attenuates the phase-shifting effect of an acute increase in activity on the circadian rhythm of activity in hamsters. These results also indicate that RO-15-1788 has an inhibitory effect on the amount of increased activity that normally occurs after transfer to a new cage with a running wheel.


Modern chronopharmacology involves the study of the effect of biological timing on the affinity and response of an organism to chemical agents. The present study investigated the behavior following nocturnal (2000-2400 hrs) or diurnal (0900-1400 hrs) administration of fentanyl to rats.

Male rats (Hw/dose) were treated with fentanyl intranasal administration (0.005 mg/kg) was approximately 3 times more potent than when given orally, which was administered diurnally (SD05 = 0.0175 mg/kg). In addition, the duration of LOR at night (2.68 min) was over 3 times shorter than when given during the day (8.76 min). The recovery index, assessed with a rotarod, indicated that the nocturnal recovery (31.3) was faster than the recovery during the day (18.2). Preliminary biochemical analysis (cAMP vs. day) has revealed a trend difference in kappa binding, while mu and delta binding remains to be reconciled. These results may have implications in the clinical use of opiates.


It is well known that muscarinic receptors regulate the onset of REM sleep. Recent findings from our laboratory indicate that REM sleep in cats can be elicited by M1 muscarinic receptor stimulation in the medial pontine reticular formation. The present experiment was designed to assess the participation of M1 receptors in the regulation of REM sleep. Sprague-Dawley rats that were chronically implanted for sleep recordings were injected in a randomized fashion with five doses (0.0, 0.5, 1.2, and 4 mg/kg) of trihexyphenidyl, a selective M1 antagonist or scopolamine, a non-selective muscarinic antagonist. There was no significant change in sleep latency by either drug. Following all doses of scopolamine, a significant increase in REM latency, as defined from time of sleep onset, was observed. However, a significant increase in REM latency was observed following the highest two doses of trihexyphenidyl. Additionally, the preliminary effects of biperiden (2.4, and 8 mg) in human subjects suggest that M1 receptors exert no influence on REM sleep. These preliminary findings in conjunction with our cat data support our hypothesis that M1 receptors do not play a significant role in the onset of REM sleep.

514.6 OLFATORY BULBECTOMY LENGTHENS TAU IN MALE SYRIAN HAMSTERS. David R. Pieper, Melinda Thompson*, and Catherine Lo¬
bbeck*. Providence Hospital, Department of Physiology, Southfield, MI 48037.

Olfactory bulbectomy (BX) increases gonadotropin secretion in male hamsters. BX also lengthens the free-running period of locomotor activity (tau) in rats and mice. It is possible that a longer tau could be causally related to the increase in gonadotropic release. The present study examined whether BX lengthens tau in hamsters. Twenty-three-day old hamsters were BX or sham (SH) and one half of each surgical group was placed in cages with exercise wheels (EX group) while the other half was housed in similar cages without wheels (SED). At this time all animals were single housed. Eight weeks later, all animals were transferred to an animal room on constant darkness (DD) in order to assess tau activity. Tau rhythms were monitored with an Estaline-Argus recorder. While the animals were still on 14L:10B, BX delayed activity onset in relation to lights off from 15 ± 0.004 hr to 37 ± 0.025 hr (p < 0.001). When the animals were placed on DD, the mean tau was 24.08 ± 0.04 hr in the SH animals compared to 24.41 ± 0.03 hr in the BX group (p < 0.001).

In conclusion, the olfactory system may be involved in the circadian clock of hamsters so that the period of the clock is longer. It remains to be determined by what mechanism or neural pathway this influence is mediated, and whether the longer tau is involved in the effect of BX to increase gonadotropin secretion.
BIOLOGICAL RHYTHMS AND SLEEP IV

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

114.7 INTRACELLULAR RECORDINGS FROM TUBEROMAMMALIAN NEURONES IN AN ISOLATED AND PERFUSED WHOLE BRAIN OF GUINEA-PIG IN VITRO.
A. KATER, M. SeraFIn AND M. MUSHELlERT. Dept. of Physiological, CMU/ENGene 4, Switzerland

Following extracellular recordings in behaving cats it has been suggested that histaminergic neurons from the tuberomammillary nucleus (TM), which have widespread projections in the brain, play an important role in the control of arousal (Lit et al., Brain Res., 1985, 479: 235-240). Recent work to survive well in an explant of the basal hypothalamus (Haas and Reiner, J. Physiol., 1988, 393: 633-646) and to resemble in many respects other amineergic neurones. While this technique is useful for assessing the intrinsic properties of TM neurones (Green et al., J. Physiol., 1990, 420:149-163), it cannot resolve their synaptology, which is important in order to understand their role in the control of behavioral states. This was thus of interest to determine whether such TM neurones could survive in an isolated and perfused whole brain (IBW) of guinea-pig in vitro and in a first step to determine whether their properties would resemble those of TM neurones recorded in the rat explant. We recorded from 20 TM neurones in the IBW. As shown previously TM neurones were mainly characterized by a prolonged after-hyperpolarization and the presence of a transient rectification due to an A current. Their basic properties were (mean ± SEM): resting potential, -58.3 ± 1.23 mV (n=11); spike amplitude; 64.4 ± 1.04 mV (n=14); spike width, 2.16 ± 0.079 ms (n=13). These neurones discharged spontaneously at a rate of 13.95 ± 1.76 spikes/s (n=11). It is concluded that TM cells survive well in the IBW and that this model can be usefully applied to their further study in vitro (supported by a Swiss NSF grant no. 31-26495-87).

114.8 NEUROTRANSMITTER MODULATION OF SLEEPING IN THALAMIC INTRALAMINAR NEURONES AND ITS FUNCTIONAL IMPLICATIONS. Ann Williams and David A. McCormick. Neurornatomy Section, Yale Medical School.

The thalamic intralaminar nuclei are major regions of the neocortex and have been proposed to control in part the excitability and pattern of activity generated in thalamocortical circuits. Indeed, neuronal activity in the intralaminar thalamic nuclei is known to be fundamentally influenced by the sleep/wake cycle and modulated by sensory inputs. Although studies have suggested that intratelencephalic inputs might be involved in sleep behavior, the functional relationship between activity in these nuclei and sleep/wake states remains unclear.

Extracellular recordings revealed that at rest intralaminar neurones fire spontaneously high frequency (200-400 Hz) bursts of action potentials at a regular rate of 0.5-3.5 Hz. Local application of NA or ACh in the hypothalamus produces rhythmic burst firing and the promotion of single spike activity. In contrast, application of HT resulted in an inhibition of rhythmic burst firing without the promotion of single spike activity. Intracellular recordings reveal that these changes in firing mode are mediated by three ionic actions: 1) a slow depolarization associated with a decrease in membrane conductance; 2) a decrease in response to large hyperpolarizing current pulses, possibly mediated by an enhancement of the hyperpolarization-activated current current; 3) an abolition of the slow (Ca2+)-activated K+ current Iapp in a subpopulation of neurones. Together, these ionic actions appear to mediate the generation of rhythmic oscillations and promote single spike activity by depolarizing the neurone out of the range in which slow oscillations can occur, by reducing responsiveness to large inhibitory inputs, and by abolishing the slow afterhyperpolarization and consequently, spike frequency adaptation. These changes in the firing mode of intralaminar neurones may then prepare the cortex to receive information arising from the primary sensory nuclei.
BIOLOGICAL RHYTHMS AND SLEEP IV

S14.13
DESPRAZINE-INDUCED REM SLEEP SUPPRESSION: EVIDENCE FOR A CENTRAL, ALPHA-1, ADRENERGIC MECHANISM. R.J. Rosa, F.J. Graech, W.A. Bell, I.D. Sanford and A.B. Morrison, Dept. of Psychiatry, University of Miami, Miami, Florida, USA 1975.

Acute noradrenaline (NE) uptake blockade by desipramine (DMI) suppresses REM sleep (REMS) in the cat and other species. We studied the underlying adrenergic receptor mechanism(s), the effect of co-administering the α1 antagonist prazosin or the β antagonist propranolol with DMI. Prazosin was ineffective. Four cats were implanted with EEG, EOG, and a frontal unilaterally electrodes. Five-hour polygraphic recordings were obtained (baseline, saline, and at 0.1, 0.3, and 1.0 mg/kg i.p. saline). REM sleep was suppressed by DMI, with 0.1 mg/kg, 1 mg/kg, and 3 mg/kg i.p. suppression of REMS by 90%, 85%, and 75%, respectively. Prazosin did not alter REMS suppression. These results are consistent with the findings in narcoleptic dogs that melanocortin antagonist, which is comparable to DMI in rats, decreases spontaneous REMS by 1%.

S14.16
SENSITIVITY AND SELECTIVITY OF IN VITRO SEROTONERGIC RECEPTOR MECHANISM IN THE MAMMALIAN SUPRACHIASMATIC CIRCADIAN CIRCUIT. E.A. Prosser, J.D. Miller and H.C. Heuser, Dept. Biological Sciences, Stanford University, Stanford, CA 94305.

The suprachiasmatic nuclei (SCN) contain a circadian oscillator that regulates temperature, circadian rhythms, and rhythm of sleep. We studied the serotonin receptor antagonist, Yohimbine (YOH) on the SCN slice preparation to investigate the role of the serotonergic systems in the SCN. YOH inhibited serotonin receptors and decreased the activity of single SCN neurons. The activity of single SCN neurons was reduced by YOH in a dose-dependent manner. The SCN slice preparation is sensitive to YOH, which may be used to study the role of the serotonergic system in the regulation of sleep.

S14.14
EFFECTS OF p-CHLOROPHENYLALANINE ON THERMOREGULATION AND SLEEP IN RATS AT SEVERAL AMBIENT TEMPERATURES. H.L. and E. Satloff, Psychology Dept., Univ. of Illinois, Champaign, IL 61820.

p-Chlorophenylalanine (PCPA), a relatively specific serotonin depletor, has been reported to cause hypoinsomnia for several days in the dark. T. Ta and H. Ta studied the participation of serotonin in the relationship between thermoregulation and sleep/waking in a 12:12 light/dark photoperiod. Thermoregulation and body temperature were measured in anesthetized rats (B6); 30°C (20°C); 0°C; 3°C (2°C). In the first 6 hr post-PCPA, the amplitude of the Tb rhythm in the first 6 hr was 1°C. At 20°C, Tb dropped 2°C in the first 6 hr post-PCPA, and the amplitude of the Tb rhythm in the first 6 hr was 1°C. In the light, Tb sleep was depressed immediately and remained lower than normal for 3 days. Sleep-wakefulness patterns changed during the first 6 hr post-PCPA, and the changes were more pronounced in the first 6 hr post-PCPA.

S14.15

Changes in serotonin (5HT) system in the anterior (NPO) and posterior (HYP) hypothalamus have been studied in relation to sleep (S) and wakefulness (W) in vivo. Vomitory and polygraphic recordings from the HYP, performed simultaneously in freely moving rats by means of a telemetry system applied to a rhythm system, were used to study the changes in serotonin. The extracellular levels of the 5HT metabolite 5-hydroxyindolacetic acid (SHIAA) significantly increased with the maximal increase at 0.5 to 6 hr after MPA and at 15-24 hr in L/H and decrease with S (slow wave and paradoxical S). Maximal increase: 15-24 hr in L/H and decrease with S. Bilateral lesions of the NPO induced an increase in W between the dark periods. Bilateral lesions of the HYP in sleep control in relation to the light-dark cycle.

S14.17

The sympathetic nervous system, which regulates not only feeding behavior but sleep, metabolism and energy balance, appears to be related to the obesity Zucker rat (Holt and York, Brain Res. 481:106-112, 1989). Zucker rats were implanted with EEG and EMG electrodes and monitored for sleep-wake pattern before, during and after the infusion of the α2-adrenergic antagonist, yohimbine (YOH). YOH was infused continuously for 5 days at a constant rate of 100 µg/kg/day (N=5, 578g) and obese (N=6, 743g) Zucker rats, using miniosmotic pumps. Total sleep time % increased significantly (26%) in the obese rat during YOH and remained elevated following YOH withdrawal. Rapid Eye Movement (REM) changes during YOH in both groups, however, YOH withdrawal caused a 30% and 39% increase in REM% in lean and obese rats, respectively, due to a 60% increase in REM period frequency. YOH and its withdrawal were associated with a significant rise in both Non-REM and REM sleep in the obese rat, this effect was evident to a lesser extent during YOH withdrawal in the lean rat.
154.19
RAPID GONADAL RECRUDESCENCE AND BODY AND LIPID MASS INCREASES WITH HYPOTHALAMIC LESIONS IN PHOTOREGRESSED SIBERIAN HAMSTERS. M. P. Mahara,* T. G. Youngstrom and T. J. Bartness, Dept. of Psychology and Biology, Georgia State University, Atlanta, GA 30303.

We previously found that combined pinealectomized (PINX) and SCNx lesioned (SCNX) photoregressed hamsters had recrudesced testes within 5 wks, but not intact or PINX-only controls. The purpose of the present experiment was to determine if a similar result was simply due to damage of retinal-photic circuitry components. Photoregessed hamsters were PINX, SCNX, given paraventricular nucleus lesions, or left intact. Blood was sampled weekly for 5 wks at which time testes and epididymal fat (EFAT) were harvested, circadian rhythms of wheelchair-running assessed and lesion histologically confirmed. Hamsters with PVNx hits and SCNX meses (mostly caudal) and dorsal to the SCN had increased testes, EFAT and body weights, increased food intake, normal activity patterns, and progressively increasing and marked serum PRL, but not FSH levels. In contrast, PINX, SCNX hits, PVNx misses and intact controls had typical short day values and SCNX hits had arrhythmic activity patterns. These results suggest an area caudal and dorsal to the SCN, and extending to and including the PVN, is involved with maintaining short day responses under these conditions and may also inhibit PRL release in long photoperiods based on preliminary lesion data.

154.20
COMPARISON OF EFFECTS ON SLEEP OF ADENOSINE AND ADENOSINE ANALOGS MICROINJECTED TO THE STRIATUM AND PREOPTIC AREA OF RATS. S.R. Ticho, M. Lekoe*i, C. Vigore'ic, E. Dziennik*, and M. Radulowich, Dept. of Pharmacology, University of Illinois, Chicago, IL 60612.

Adenosine A2 receptors are located in the olfactory tubercle, nucleus accumbens and striatum while adenosine A1 receptors show a heterogeneous distribution throughout the CNS. To localize adenosine's behavioral effects within different brain areas, we compared the effects on sleep of adenosine and adenosine analogs microinjected to the striatum and preoptic area of the rat. Polygraphic recordings were examined during a six hour period of the light cycle. Microinjections to the preoptic area were 0.5ul while injections to the striatum were 1.0ul. All three of the drug treatments, ADO (0.025M, n=10), a selective ADO A1 receptor agonist (CPA, 0.001M, n=5), and a nonselective ADO A1/A2 receptor agonist (NECA, 0.002M, n=7) microinjected to the preoptic area increased deep slow-wave sleep (SWS2) (27%, 27%, 48%) (p<0.05) respectively as compared to saline controls. Furthermore, ADO and NECA caused a significant increase in total sleep (13%, 16%) (p<0.05). In contrast, no changes in sleep parameters were observed when these drugs (ADO, n=6) (CPA, n=6) (NECA, n=6) were microinjected to the striatum. We observed a 20% increase SWS2 with NECA, this increase did not attain significance.

154.21

Muramy peptides (MP) enhance sleep in experimental animals. Muramyl dipeptide (MDP; N-acetyl-muramyl-L-alanyl-D-isoglutamine, originally synthesized as an immuno- stimulant, is also somnogenic and pyrogenic. Additionally, a MP is the active component of Urinary Sleep Factor S. FK-156 (L-tyro- nyl-D-glutamyl-(L)-meso-diaminopimelic acid) and FR-40929 (L-tyro- nyl-D-glutamyl-(L)-meso-diaminopimelic acid) are MP analogs which lack a sugar moiety. FK-156 is a potent, while FR-40929 is a weak, immunostimulant. In order to further understand the structural requirements for different pharmacological activities we compared the somnogenic and pyrogenic effects of FK-156, FR-40929 and MDP in rabbits. MDP increased slow-wave sleep (SWS) and body temperature (BT) at doses of 25-100/µg/kg s.c. FK-156, 100 µg/kg s.c., induced changes in SWS and BT that were comparable to 100 µg/kg of MDP. In contrast, FR-40929 was less potent than MDP and FK-156, with a threshold dose for increasing SWS and BT of 0.3 mg/kg s.c. These data indicate that a sugar moiety is not necessary for the somnogenic and pyrogenic effects of MP analogs.

NEUROTOXICITY: MPTP

155.1

In rodents and primates methamphetamine (METH) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) are known to be toxic to proapoptotic dopamine (DA) terminals. This study was designed to evaluate the in vivo effects of toxic doses of METH and MPTP on monoamine levels and 3-methyl-D-aspartate (NMDA) and phencyclidide (PCP) receptors in mouse brain. Adult male C57/SH mice were injected four times with 5 or 10 mg/kg METH, i.p. at 2 hr intervals or single injections of 0 or 25 mg MPTP/kg, i.g. Animals were sacrificed 3 days Later. NMDA (+Glu catalyst) and PCP (-TCP) receptor binding were analyzed in cerebral membrane preparations using filtration techniques and monoamine concentrations in striatum by HPLC/EC. Dopamine levels in striatum were significantly decreased (40-50%) after METH or MPTP treatment. In vivo METH and MPTP significantly decreased PCP but not NMDA receptor binding. MPTP (25mg/kg) produced a concentration dependent decrease in PCP and NMDA receptor binding but METH produced a decrease in PCP binding only at high doses (1000 µM). These data demonstrate that neurotoxicity induced by METH or MPTP, as indicated by decreasing DA levels, results from reduced ligand binding to the PCP or NMDA receptor complex.

155.2

Methamphetamine is a potent dopaminergic neurotoxin in the nigrostriatal tract of mice. This neurotoxicity is dependent on dopamine synthesis and release and is blocked by dopamine receptor antagonists such as haloperidol and by the non-competitive NMDA receptor antagonist MK-801. It has previously been reported that methamphetamine pretreatment leads to partial protection against a subsequent challenge dose of methamphetamine. In the present study, we have evaluated the effects of various pretreatment regimens on methamphetamine- and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurotoxicity. Mice were pretreated with methamphetamine or cocaine for four days and challenged on the fifth day with either methamphetamine or MPTP. Dopaminergic neurotoxicity was evaluated several days later by quantifying neonatal dopamine content and tyrosine hydroxylase activity. A low dose pretreatment regimen with methamphetamine led to a nearly complete protection against subsequent methamphetamine- induced neurotoxicity. In contrast, cocaine pretreatment potentiated both methamphetamine- and MPTP-induced neurotoxicity. Potential mechanisms for these observations will be discussed.
515.3

DOPE N-oxide is the major peripheral metabolite found in vivo following systemic injection of the dopaminergic neurotoxin, MPTP (Lau et al., Life Sci. 52(1998), Chiba et al., J. Neurochem. 74(1998)). Therefore the presence and high concentrations of this potential neurotoxic metabolite have not been described. MPTP N-oxide (15 µg/side) or saline was injected bilaterally to the caudate-putamen of male C57/BL mice. Five hours later, the striatal dopamine (DA) levels in mice treated with MPTP (8±1.5 mg/kg) and MPTP N-oxide (11±1.1 mg/kg) were significantly lower than in the control mice (15±0.6 mg/kg). In vitro incubation of striatal homogenates with MPTP or MPTP N-oxide (0.89 mM) for 5 hours revealed that there was a time-dependent conversion of MPTP to MPTP

515.4

DOSE-DEPENDENT EFFECTS OF 2-CH3-MPTP ON MONOAMINERGIC SYSTEMS IN MICE: TYROSINE HYDROXYLASE AND GFAP IMMUNOCHEMICAL STUDIES. D. L. Young, L. L. Chen, and M. Gupta, Dept. of Anatomical and Neurological Sciences, Univ. of Louisville, Louisville, KY 40292.

Previous studies from this laboratory have shown that 2-CH3-MPTP produces a significant decrease in the number of tyrosine hydroxylase (TH)-positive neurons in substantia nigra and the ventral tegmental area. These studies were undertaken to investigate if additional monoaminergic nuclei including locus coeruleus and the dorsal raphe dopaminergic neurons are also affected and if these effects are dose-dependent. Young adult male C57BL/6 mice were given multiple injections of 2-CH3-MPTP (7.5 and 10 mg/kg) over a two day period. Three days later, control and treated mice were anesthetized and perfused with the fixative. Adjacent 40-µm thick serial sections through the brain were stained immunocytochemically for TH and GFAP. The number of TH-positive neurons were quantitated in the SN, VTA, A8, locus coeruleus and dorsal raphe as well as GFAP-immunoreactive astrocytes in the striatum. The results show that 2-CH3-MPTP produced a statistically significant and a dose-dependent decrease in the number of TH-immunoreactive neurons in the SN, VTA and A8 followed by extensive gliosis in the striatum compared to the controls. These data demonstrate that 2-CH3-MPTP is much more toxic than MPTP HCl and affects several monoaminergic nuclei in the brain. Quantitative analysis on gliosis in the striatum is currently in progress. Supported by USPHS grants R29 NS24291 to MG.

515.5


Immunohistochemical staining with Mac-1 antiserum reveals microglial cells. The cells are numerous and located in gray and white matter in all areas of the brain. Cell bodies are small, processes are elongated and spined, and appear like microglial cells after silver carbonate staining. After IP 1-methyl-4-phenyl-1,2,3,6-tetrahydroisoquinoline (MPTP) (15 mg/kg s.c. for 2 days, 3 days after last injection), Mac-1 staining reveals very intensely stained cells accumulated in the nucleus accum-bens and substantia nigra in the substantia nigra and the putamen compacta. These areas of dopaminergic cells, fibers, and terminals, and this can serve as an illustration of the specificity and sensitivity of MPTP as a dopaminergic neurotoxin. These cells appear to be macrophages and have stained cell bodies and numerous short processes radiating out from the cell body. Since both microglia and macrophages have the same antigen type, we suggest that resident microglia inherent in the brain become reactive microglia after MPTP on dopaminergic neuronal elements, and hypertrophy, become round, shorten their processes and become macrophages. The mobilization of macrophages after MPTP adds an additional factor to the etiology of the pathophysiology of MPTP-induced neurotoxicity.
THE METABOLIC EFFECTS OF MMP+ IN CULTURED CEREBELLAR GRANULE CELLS A.M. Martin, T.S. Nowak and J.L. Kopin.
Clinical Neuroscience Branch, NINDS, NIH, Bethesda, Maryland 20014.
We have determined, in a homogenous population of cerebellar granule cells in culture, intracellular metabolite levels of adenosine triphosphate (ATP), phosphocreatine (PCr) and creatine, as well as medium glucose and lactate levels. When exposed to 50 micromolar MMP+, MMP+ depleted PCr to 20% of control levels within 30 minutes and it remained at this level for up to one week. The decrease in PCr was accompanied by an equimolar increase in creatine. No significant change in ATP levels occurred within the first 48 hours, but 50% decreases were observed after this time. There was also a marked decrease in medium glucose, with enhanced lactate accumulation. Neurons remained viable for at least 9 days in culture. Although glucose was repleted every three days after day 7 in culture, neuronal death occurred after day 12 in culture. These results suggest that the neurons are retaining their ATP levels through anaerobic metabolism of glucose in the presence of MMP+. 

Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.
1-Methyl-4-phenylpyridinium ion (MPP+), a major brain metabolite of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, is an inhibitor of complex I of the mitochondrial respiratory chain. We have synthesized several analogs of MPP+ containing various alkyl groups in the 4'-position of the phenyl ring and have determined their capacity to inhibit the oxidation of NADH-linked substrates by intact mouse liver mitochondria. The analogs are potent inhibitors of respiration than MPP+ itself, with the potentials being as long as the length of the 4'-alkyl chain increases. The most potent inhibitor, the 1-methyl-4-((4-hexylphosphoryl)phenyl)imidazole (HPI), was about 200 times as effective as MPP+ itself (IC50 < 100 μM). In addition, tetrahydrobornyl (TBP), a lipophilic analog, was able to increase the potential of all of these derivatives. However, the relative degree of potentiation appears to be inversely proportional to the length of the 4'-alkyl chain increases. Thus, all of the MPP+ analogs are closer in inhibitory potency in the presence of TBP than in their absence. These observations suggest that the presence of an aliphatic 4'-alkyl group increases the accessibility of the compound to its inhibitory site. These analogs should prove to be useful tools for studying the nature of the process whereby MPP+ and its pyridinium analogs interact with complex I to inhibit mitochondrial respiration.

California Parkinson’s Foundation and California Institute for Medical Research, San Jose, CA 95128.
Incubation of mouse brain synaptosomes (essentially devoid of contamination by extrasympatoplasmic mitochondria) in the presence of MPP+ revealed a concentration-dependent and accumulation. Intrasympatoplasmal concentrations of 79 μM and 106 μM were reached 10 and 30 min, respectively, after addition of 50 μM MPP+. The uptake of MPP+ into synaptosomes was relatively unaffected by ouabain (a NaK pump inhibitor) or by ouabain (a K+ pump inhibitor). In contrast, the rate of MPP+ uptake was greatly increased by tetraphenylborate, a lipophilic anion that facilitates the transport of pararomatic cations across the plasma membrane, by a Na gradient concentration gradient. Furthermore, MPP+ accumulation was significantly increased (by substituting NaSCN and KSCN for NaCl and KCl in the perfusion medium) by (a) ouabain (a NaK pump inhibitor) or by (b) ouabain (a K+ pump inhibitor) as a consequence of enhancing or lowering, respectively, the plasma membrane potential of synaptosomes. Data indicate that (1) MPP+ present at concentrations in the 10-20 M range can cross neuronal membranes despite its charged chemical structure and without the need of a specific uptake mechanism, and (2) polarization of neuronal membranes may facilitate the accumulation of this toxic cation into nerve terminals.

MITOCHONDRIAL RESPONSE IN N2A-1 NEUROBLASTOMA CELLS AFTER EXPOSURE TO MMP+. S.J. Simmons, J.F. Hansen and M.T. Statler.
1Environmental Health Science Center and 2Department of Neurology and Anatomy, University of Rochester, Rochester, NY 14627.
The effect of MMP+ on the N2A-1 mouse neuroblastoma cell line was examined by electron microscopy with morphometric analysis and by the metabolic parameters glucose consumption and lactate production. It was demonstrated that differentiated N2A-1 cells are less sensitive to MMP+ toxicity than mitotic N2A-1 cells as assessed by morphology, cell number and protein incorporation.
Mitochondrial area, ratio of mitochondrial area to cytoplasmic area (M/Ca), and percent damaged mitochondria were measured morphometrically. The control mitotic and differentiated N2A-1 cells were compared for all three parameters. After MMP+ (100μM) exposure for 24h, the Ma/Ca increased from 9% to 15%, the mitochondrial area tripled, and 85% of the mitochondria were damaged in both mitotic and differentiated cells. Mitochondria of both mitotic and differentiated cells were equally affected by MMP+ exposure.
Lactate production and glucose consumption were 300% of control after 24h MMP+ exposure in both mitotic and differentiated cells. After 48h exposure, total lactate production increased by 200% of control in both phenoxyphine. A similar response was seen after exposure to the mitochondrial toxin Rotenone, indicating a loss of mitochondrial function and increased dependence on glycolysis for energy.
These data indicate that while mitotic N2A-1 cells are more sensitive to MMP+ in whole cell response (cell death and decreased protein synthesis), the mitochondria of both mitotic and differentiated cells respond equally to the toxic insult, suggesting that the biochemical requirements for ATP production are similar in the two cell lines.

Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.
The dopaminergic neurotoxicity of MPP+ and several of its analogs depends on (1) bioactivation of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine to a pyridinium species via MAO and (2) active accumulation in dopaminergic nerve terminals into the dopamine uptake system. Additionally, evidence exists which suggest that the inhibition of mitochondrial oxidation of NADH-linked substrates by these pyridinium species is an important feature of the neurotoxicity. In this study, several compounds were tested for their capacity to act as substrates for the dopamine transporter and for their ability to inhibit mitochondrial respiration. Compounds were subsequently chosen for in vivo neurotoxicity studies which demonstrated ranges in potency to serve as a substrate for the dopamine transporter and to inhibit mitochondrial respiration. The neurotoxicity of MPP+ and its analogs was determined after the central administration of the compounds via an in vivo dialysis probe followed by the measurement of stimulated extracellular dopamine levels. Neurotoxic MPP+ analogs, like MPP+ itself, caused an irreversible depletion of DA as demonstrated by the lack of a stimulated increase in extracellular DA. This was independent of an infusion of MPP+ 24 hours after an initial exposure to the analog. Data from the in vivo dialysis studies strongly support the theory that the neurotoxicity of these compounds depends not only on their ability to act as substrates for the DA carrier but also on their capacity to inhibit mitochondrial respiration.

MPP+-TYPE NEUROTOXICITY OF A PYRIDINIUM METABOLITE DERIVED FROM HALOPERIDOL. L. Rallena, B. Subramanyam* and A. Castagnoli*.
Dept. Medichal Chemistry, University Groningen, 9713 AW The Netherlands and Dept. Chemistry, Virginia Polytechnic Institute, Blacksburg, VA 24061.
Neurotoxic properties of a recently described (Subramanyam, B. et al. BRLC, 166:238, 1990) pyridinium metabolite (HALP+) of the neurolepetic anti agent haloperidol, were compared with those of the dopaminergic neurotoxin MPP+. A rat intrastriatal microdialysis assay was employed to assess the potential toxicity of HALP+. The test compounds (2mM MPP+ and 2mM HALP+) were infused intrastriatally via the dialysis probes for various time periods and the output of the neurotransmitters and metabolites under investigation was monitored continuously. A challenge perfusion with MPP+ 24 hours later was used to determine the extent of neurotoxicity produced by the microdialysis. Preliminary results indicate that the rat haloperidol and HALP+ with the test compounds the previous day. HALP+ lacks the acute potent dopamine (DA) releasing effect of MPP+. However, a 7.5 hour lasting HALP+ perfusion compensates dopamine release shown by a small DA release induced by the challenge MPP+ perfusion. The effects of HALP+ on lactate production were also measured by microdialysis. A 1 hour perfusion with HALP+ caused an increase in lactate levels to about 150% of control indicating that HALP+ like MPP+, inhibits mitochondrial respiration in vivo. These results show that various other types of quaternary pyridinium compounds, not closely structurally related to HALP+, also suggest a potential role of HALP+ as a cause of persistent tardive dyskinesia after chronic use of haloperidol. Supported by NS306 and NATO CRG90573.
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

1280

NEUROTOXICITY: MPTP

THURSDAY PM

515.15 TRANSGENIC MICE EXPRESSING THE HUMAN SOD GENE ARE RESISTANT TO MPTP-INDUCED TOXICITY. V. Prebodka, V. Kostic, V. Jackson-Lewis, R. Carton, P.J. Epstein, J.L. Cited (KUMTA. Columbus University, New York, NY 10032 and University of California, CA 94134). MPTP causes significant depletion of striatal dopamine (DA) which is similar to that seen in Parkinson's disease (PD). Free radical (FR) toxicity has been suggested to be involved in the pathogenesis of both PD and MPTP toxicity. Superoxide dismutase (SOD) is a key enzyme of the antioxidant system that protects cells from the hazards of FR. In order to further evaluate the role of FR in MPTP-induced toxicity, we tested the possibility that transgenic (TG) mice expressing the human SOD gene (Epstein et al., PNAS 84:8904, 1987) might be protected against the toxic effect of MPTP. Young white adult SOD-Tg mice and their non-Tg littermates were injected with MPTP (30 mg/kg, i.p. for 3 days) while SOD-Tg and non-Tg control mice received saline injections. Five days after the last injection, the mice were sacrificed and striatal, scrotum (5-HT) and their metabolites (DOPAC, HVA and 5-HIAA) were determined. MPTP reduced DA level (%67, P<0.05) in the non-Tg mice. DOPAC and HVA levels were also significantly decreased. In contrast, MPTP did not affect DA, DOPAC, or HVA levels in the SOD-Tg mice. In both MPTP-treated groups, 5-HT and 5-HIAA levels were not different from controls. These results indicate that increased SOD activity may prevent the toxicity of MPTP in mice presumably by scavenging FR formed after MPTP administration. NCRRHD HD-17035, PDR.

515.16 ACUTE MPTP INDUCES C-FOS GENE EXPRESSION IN MOUSE BRAIN. A.M. Duchemin, P.K. Gudelishtv, N.H. Neff and M. Hadjiconstantin, Depts of Pharmacology and of Psychiatry, The Ohio State University College of Medicine, Columbus, OH 43210. c-Fos protein acts as a transcriptional regulatory factor for a number of genes expressed in the brain. Its synthesis can be increased by various stimuli that modify neuronal function. In this report, we show that the neurotoxin, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), induces a transient expression of c-fos gene in the mouse brain. MPTP was injected into mice and total RNA from several areas of the brain extracted at different time intervals following the injection. c-fos mRNA was assayed by Northern blot hybridization with a nick-translated Xho-I-Ncol restriction fragment from the pc-fos (mouse) 3' gene obtained from ATCC. c-fos mRNA increases were detectable with doses of MPTP as low as 10 mg/kg and the changes increased with the dose of MPTP. The dose-related changes were similar for several brain regions. The time-course of the induction of c-fos mRNA was studied after a dose of 50 mg/kg MPTP. In the striatum, c-fos mRNA increased 20 min after injection, was maximal at 40 min and decreased to control values at 90 min. In the hippocampus, the increase of c-fos mRNA was delayed: the content was maximal at 50 min, remained elevated at 90 min and decreased to control levels at 2 hrs. The D1 and D2 dopaminergic receptor antagonists, haloperidol (10 mg/kg), sulpiride (150 mg/kg), and SCH 23390 (10 mg/kg), injected i.p. 30 min before MPTP, did not prevent the increase of c-fos mRNA. These results suggest that the response to MPTP is apparently unrelated to interaction with dopamine receptors.

515.17 EARLY-LIFE MPTP EXPOSURE SLOWS AGE-RELATED LOSS OF SUBSTANTIA NIGRA NEURONS. W.G. Tatton, C.E. Greenwood, N.A. Senik, P. Sato, D. Holland*, and M. Kwart. Departments of Physiology and Nutritional Science, University of Toronto, Toronto, Ontario, Canada. MSS 1A8. We showed that four populations of immuno-cytochemically identified mono-amnergic neurons display different rates of age-related loss across the lifespan in C57Bl mice (Tatton et al. Soc. Neurosci. 15:160-189, 1989). Neuronal loss was exponential (% Loss = e^-AtAge in wks + 8) with 90% confidence limits from 1 to 3%. Calne & Langston postulated that toxin-induced and age-related neuronal loss sum to determine the time course of disabilities found in some neurodegenerative diseases (Lancet 2:1457,1983). We tested this hypothesis by treating C57Bl mice with 60 or 150 mg/kg of MPTP or saline at 8 wks of age and examining substantia nigra compacta (SNc) neurons at 52 or 88 wks of age using immuno-cytochemistry for tyrosine hydroxylase (TH), neuron specific enolase and neurofilament proteins together with the retrograde transport of fluorogold. Measurement of the % area of TH+ tissue in the ipsilateral striatum normalized against the number of TH+ SNc somata (counted from alternate sections through the whole nucleus) was used as a measure of relative terminal axonal length. Comparison of the numbers of surviving TH+ SNc neurons in MPTP treated mice to summed values for the age-related loss plus the MPTP dose-dependent loss of TH+ SNc neurons at 20 wks after MPTP exposure (Senik et al. Br. Res. 1990, in press) showed that MPTP treatment at 6 wks of age markedly slows the rate of loss of neurons surviving MPTP exposure (a constant = 0.0130 for saline but = 0.0068 for SNc neurons surviving early life MPTP p< 0.001). Saline-treated and MPTP-treated age-matched neuron showed average increases of somal crosssectional area, estimated terminal axonal length/SNc neuron and TH immunodensity/L unit area of soma relative to those in 8 wks old mice that are proportional to the combined toxic loss of neurons by toxic exposure and aging. The slowed age-related loss of SNc neurons surviving early-life MPTP exposure will be considered relative to axonal sprouting and attenuation in the synthesis of specific proteins in the neurons. (MRC Canada grant MT5218)

516.1 A COMPARISON OF SPATOTEMPORAL VOLTAGE DISTRIBUTIONS OF THE SECOND AND THIRD DIVISIONS OF THE TRIGEMINAL NERVE EVOKED POTENTIAL IN MAN. C.G. Widmer. Dental Research Center, Emory University School of Dentistry, Atlanta, GA 30322. Mapping the initial cortical response to trigeminal nerve stimulation has been reported (Widmer, C.G., J. Dent. Res. 66:117, 1987), but no study has examined spatiotemporal voltage distributions of different divisions within the same subject. Also, no study has mapped the muscle reflex potentials which may contaminate the evoked response. The purpose of this study was to map the evoked responses after stimulation of the great auricular nerve (V1) and mental nerve (V3). Median nerve evoked responses were used as a control. Three subjects were stimulated with a 0.2 ms pulse at 1.2 Hz and averaged for 512 stimuli. Potentials from 28 scalp recording electrodes were amplified (band-pass 1-1000 Hz), acquired at 4000 Hz/channel, averaged by a computer acquisition system (BrainWave Systems) and stored on disc for subsequent mapping. Topographical activity maps were generated (BrainWave Systems) in the range of ±20 ms post stimulus. The maps were normalized and 4.0 ms for the trigeminal nerve and the initial cortical responses were identified and latencies were recorded. Median nerve evoked responses occurred at 20.2 ± 1.4 ms with the greatest activity in the parietal region contralateral to the stimulation site. Both V1 (12.1 ± 1.0) and V2 (12.8 ± 1.1) distributions were highest in amplitude over the temporal region of the somatosensory cortex (C3-T3, C6-T4) contralateral to the stimulation site. The V2 distribution was located slightly superior to the V1 initial cortical response consistent with known topographical distribution in the somatosensory cortex. Muscle reflex potentials were identified ipsilaterally in the same region. Supported by USPHS DE 06974.

516.2 FRACTAL DIMENSION OF AN EVOKED POTENTIAL. W.D. McCall, Jr. and C.G. Widmer. SUNY Buffalo, NY 14214 and Emory Univ., Atlanta, GA 30322. If the evoked potential is deterministic and chaotic, it should have a fractal dimension. Our purpose was to obtain the fractal dimension of an evoked potential. The median nerve in one subject was stimulated at 1.2 Hz with 0.2 ms pulses at 3 times per second (28 ms intervals) while recording each response from 28 sites simultaneously. The correlation dimension (Cox & Lacasa, Proc. Soc. Exp. Biol. & Med. 1983) was calculated for the 30 ms potential using recording sites as embedding dimensions. Straight lines were fit to the log-log display of the cumulative distribution of distances by a least-square-error algorithm. An acceptable fit had five or more points and a correlation coefficient of 0.980 or greater. The correlation dimension increased until the 12th embedding dimension where the correlation dimension was 6.4. Since the triboelectric Poincare section reduced the original dimension by one, we interpret our result as suggesting that the fractal dimension of the evoked potential was about 7.4. Supported by DE-07089 and DE-06974.
S16.3 NEUROLOGIC EFFECTS ON HUMAN MIDLATELLATION AUDITORY EVOKED RESPONSES. R.J. Strandburg, J.S. Buchwald, E.H. Rubenstein* and J. Schwartz*. Depts. of Psychiatry, Physiology & Anesthesiology. UCLA School of Medicine, Los Angeles, CA 90024.

In contrast to the Pa component of the auditory midlatency evoked response (MLR), marked "P" abnormalities are observed in Alzheimer's disease, autism and schizophrenia. To further differentiate and characterize these two components, click elicited MLRs were recorded from normal malevolunteers receiving the cholinergic antagonist scopolamine followed by the agonist physostigmine. Pa (25-60 msec). Nb (40-80 msec) and P1 (50-65 msec) components, obtained in these 2 conditions at a central scalp lead (C1) were compared with immediately preceding control data recorded with click rates of 1, 5, 8 and 10/sec (P1 disappears at the faster rates while Pa is not affected).

Intavenous scopolamine eliminated the Pa and slightly increased Pb subjects reported drowsiness but were awake with eyes open throughout the recordings. Subsequent injections of physostigmine resulted in a rapid reversal of the scopolamine effects; the subjects became alert. Pa decreased, and P1 returned to its control amplitude (and declined in response to rapid click rates). These data in conjunction with the results of related studies of MLRs in the cat suggest that the Pa generator system includes a cholinergic brainstem-thalamic component of the ascending reticular activating system.

(Supported by USPHS Grants HD00958 and HD04612)

S16.5 CORRELATION OF DICHTIC LISTENING WITH CORTICAL AUDITORY EVOKED POTENTIALS IN PATIENTS WITH COMPLEX PARTIAL EPILEPSY. S.Khosbin, S.J. Murawski and C.S. Petrou*. Dept. of Neurology, Harvard Medical School, Boston, MA 02115.

Forty patients with history of partial complex seizures were studied using the dichotic listening test (Kinsara, 1961), the Wechsler Adult Intelligence Scale, routine electroencephalography and cortical auditory evoked potentials. Correlation was found between these tests and information from neurological exams and radiographic studies. All patients with abnormal dichotic digits had abnormalities on cortical evoked potentials at N100, P200 or P300 components. The routine electroencephalogram was rarely informative. Abnormalities of Dichotic digits correlated well with topographic mapping of evoked potentials with regard to dominant and non-dominant hemisphere findings. Good correlation was also found between discrepancies in verbal and performance scores on the Wechsler Adult Intelligence Scale and cortical auditory evoked potentials. Sample cases will be illustrated.


The "cat-P1", previously called by us "wave A", is a positive potential evoked by clicks, with a latency of 20-30 msec, recorded from the vertex. Like the human P1, it is absent with rapid click repetition rates (10/sec) and during sleep, or with theophylline, but present during wakefulness and REM sleep. Both the P1 and cat-P1 are reversibly eliminated by scopolamine, a muscarinic cholinergic antagonist, and recover with cholinergic agonist nicotine. The human P1 is absent in a group of Alzheimer's disease patients, and the cat-P1 is abolished by bilateral lesions of the pons and temporal pole (RFT) and other midbrain reticular formation. To test the hypothesis that the vertex-recorded cat-P1 is generated by PFT projections to the thalamus, we recorded from thalamic sites and tested midlatency click-evoked potentials with the effects of rapid repetition rates and cholinergic drugs. EEG was recorded from awake cats with clicks presented at a rate of 0.2/sec or 10/sec. Most of the depth-recorded potentials, many targeted for thalamic nucleus centrum medianum, were diminished or abolished by rapid click rates and scopolamine. Like the vertex cat-P1, the depth potentials are sometimes recovered or were enhanced by physostigmine or carbachol. These data further support the hypothesis that the vertex cat-P1, and perhaps the similar human P1, reflects activity in a cholinergic system projecting from thalamus to the thalamus. (Supported by USPHS HD00958 and NS25400).


We stimulated distal median nerve at the wrist using monophasic magnetic coil (MC) pulses and a novel hardware switching device which reverses current in the round and figure of 8 MC (Cathwell Laboratories). Thus, no errors are introduced by physically rotating the round MC through 180° as reverse current flows. Thenar motor unit responses were elicited at threshold intensities. No shift in latency was detected when the current in the figure 8 MC was reversed (Fig. la) confirming our previous observations when the round MC was rotated. These data differ markedly from those obtained with conventional electrical stimulation where a shift of 0.4 - 0.5 msec for each anodal to cathodal anodal distance of 2 cm. A possible explanation is that current flows obliquely into and out of nearby nodes.

S16.8 HUMAN NEUROMAGNETIC AND NEUROELECTRIC ALPHA FREQUENCY ACTIVITY REACTIVITY AND EVOKED FIELDS. C.C. Galien, S. Hampson*, T.T. Yang*, F. Bloom, W. Young. Dept. of Neurophysiology, Res. Inst. of Scripps Clinic, La Jolla, CA 92037.

A "moving window" approach was used to estimate the rates and timing of stimulus-related reductions in magnetoencephalographic (MEG) and electroencephalographic (EEG) intrinsic alpha frequency power in five paired (MEG probe located directly over EEG recording) occipital sites. Serial recordings of 120 epochs of cycling visual stimulus on/off in eight normal subjects allowed isolation of the two-second periods preceding and following onset of visual stimulus in each epoch. Waveforms were rectified and the average amplitude of alpha in an initial 100 msec window was calculated and plotted. Subsequently the window was "moved" in small increments and the average amplitude serially recalculated and plotted. This process produced a smoothed line reflecting fluctuations in alpha amplitude over time. Modelling studies with square wave and linearly sloped data revealed potential distortion of latency estimates for onsets and cessation of alpha suppression. But, when certain specified characteristics were present, this measure showed utility as both a comparative measure of rate of change and for estimation of the midpoint of signal decline. As used in this study, the "moving window" method indicated maximal alpha reactivity in the first few hundred msec following stimulus onset, a period which overlapped multiple visual evoked waves. With limitations, the moving window approach also estimates the rate of decline of rhythmic activity.
516.9


Magnetic Resonance Imaging (MRI), Brain Electrical Activity Mapping (BEAM), and neurophysiological tests presumed to be mediated by the frontal lobes were administered to 41 patients with schizophrenia (n=35) or schizophrenia spectrum disorder (n=6), and to 23 normal controls. Regional Cerebral Blood Flow (RCBF) measures were also administered to 35 of the patients and 16 of the controls. BEAM and RCBF were conducted after a 21 day drug wash, or in 9 first episode patients, prior to neuroleptic treatment. MRI, BEAM, RCBF, and neurophysiological data were independently used to categorize patients into frontal lobe impaired and non-impaired groups.

Two methods were used to dichotomize the group: a purely statistical method (i.e. upper quartile of each distribution is defined as impaired), and a more clinically-based technique (e.g. significant frontal slowing on BEAM, multiple perseverative errors on Wisconsin Card Sorting Test). Bayesian probabilities are used to compare the categorization produced by the two methods of dichotomizing the patients on each of the imaging and testing procedures. Results are complex and illustrate the difficulty of defining frontal lobe impairment in disorders such as schizophrenia in which there is no obvious lesion.

516.10


Previous studies (Harter et al. and Naylor et al.) showed a left central P240 deficit in dyslexic children and a bilateral central P240 deficit in dyslexic adults, respectively, in a visual letter discrimination task. Flowers et al., showed focal left tempo-parietal rCBF increases in dyslexic adults, compared to controls, in an auditory word length analysis task.

In a sample containing 13 dyslexic and 10 non-dyslexic adults, both defined by childhood reading scores, the abnormal left tempo-parietal flow increase was correlated (p < .01) with both left and right central P240 as well as with left and right frontal P240 amplitude reductions. While this suggests convergence on a common deficit, across modalities and across physiological methods, it raises an important question of rCBF source localization which is discussed by reference to illustrative MEG findings from individual subjects.

516.11


Persons with cerebrovascular disease are at risk from the marked shifts in blood flow evoked by the Valsalva maneuver (VM) or straining. We examined the influence of age, hypertension and position on carotid artery blood flow velocity (CABFV) during strain in the 70th upright and supine bed positions. Young healthy (n=180) and hypertensive (n=252) adults aged 35-50 years and older healthy subjects (n=46, >55 years) were studied. Subjects strained by blowing into a pressure gauge to 40 mmHg for 30 seconds. CABFV was measured with a Doppler technique. In the supine position, CABFV fell an average of 59% during strain for young hypertensives (p<.05). Following release of strain, young hypertensives had significantly greater, overshoot above baseline than young healthy subjects (25% vs 17%, p<.01) and tended to have a greater overshoot than the older healthy group. During strain, supine and upright positions differentially altered CABFV in both young healthy subjects (-60% vs -46%, p<.05) and young hypertensives (-79% vs -50%, p<.001). Across the VM, total percent change in CABFV was less in the upright position vs supine for young hypertensives (69% vs 999%, p<.01) but not for controls. We conclude that CABFV changes during the VM are intensified in young hypertensives as compared to young and older healthy adults and that the intensity of these changes can be modified by body position.

*Supported by NIH, NCNR, grant # R01 NR01142-05.

516.12


Monitoring the brain during ECT has typically been restricted to the measurement of seizure duration, the most significant variable for therapeutic efficacy. With the introduction of "modified" ECT (i.e., the use of intravenous anesthetic drugs, neuromuscular blockade and continuous EEG), standing EEG now significantly involves anesthesiology. Thus, the purpose of the present study was to apply continuous monitoring of computer-processed EEG and EMG activity during ECT in order to tailor anesthetic and paralytic management to individual needs, and establish a reliable technique for real-time evaluation of seizure duration. All consenting patients met the DSM-III-R criteria for a major depressive episode (25 patients received 115 treatments). Nondominant unilateral or bilateral ECT was administered using brief bilateral pulses delivered by the Multiple-Monitor Electroconvulsive Therapy Apparatus (MECTA Corp., Northridge, CA). Two channels of analog and computer-processed EEG were continuously monitored. The latter method involved power spectral analysis and display of the EEG as a density-modulated spectral array (DSA) with trended rms amplitude and spectral edge frequency (SEF 90%), using the 2-channel SRD Cerebro-Trac 230+ (Mtsav, Israel) and a standard triaxial, frontal-mastoid electrode configuration for the left and right hemispheres. The DSA profiles demonstrated that anesthetic induction, neuromuscular blockade, ECT seizure duration and recovery from each were more easily and consistently interpreted than using analog waveforms. We conclude that computer-assisted brain monitoring is superior in conventional techniques, and offers expanded capabilities for research on ECT and for its clinical management.
S15.15

RELIEF OF SPASTICITY BY TENS IS ASSOCIATED WITH IMPROVEMENT IN REFLEX AND VOLUNTARY MOTOR FUNCTIONS. C.W.Y. Chan and M.F. Levin. School of Physical and Occupational Therapy, McGill University, Montreal, Canada, H3A 1Y5.

Our previous studies showed that single, 45 min applications of transcutaneous diadidac nerve stimulation (TENS) prolonged H and stretch reflex latencies in hemiparetic subjects. In addition, nine, daily 30 min TENS applications enhanced sensory inhibition of the H reflex and tended to decrease hyperactive stretch reflexes. These findings suggested that longer-term TENS may be effective in the reduction of hemiparetic spasticity. Our present objectives were two-fold: to determine whether longer-term TENS stimulation would lead to a reduction in spasticity and whether such a reduction could be associated with a decrease in stretch reflex excitability and an improvement in voluntary motor function.

Three patients were studied. The effects of four, daily 60 min TENS treatments over a three week period were contrasted with those of placebo stimulation applied to the common peroneal nerve of the affected leg. Ten test battery consisted of five measures of reflex and voluntary function. The effects of these four, daily 60 min TENS treatments over a three week period were contrasted with those of placebo stimulation applied to the common peroneal nerve of the affected leg. Ten test battery consisted of five measures of reflex and voluntary function.

To contrast placebo stimulation which produced no significant effect, repeated applications of TENS decreased clinical spasticity (p<0.05), increased voluntary force, and, decreased the magnitude of stretch reflexes (p<0.05) in the spastic ankle extensors. These changes occurred concomitantly with substantial improvement in voluntary dorsiflexion force (p<0.05) and a decrease in antagonist EMG co-contraction ratios (p<0.05).

Our results thus demonstrated that repeated applications of TENS can reduce spasticity and improve voluntary force and range of motion in hemiparetic subjects. Furthermore, the mechanism of the improvement may be partly related to an enhancement in presynaptic inhibition, and a possible "disinhibition" of descending voluntary commands to flexor motor neurons.

S15.16

SPASTICITY IS INVERSELY CORRELATED WITH ANTAGONIST VOLUNTARY CONTRACTION IN SPASTIC HEMIPARETIC SUBJECTS. M.F. Levin and C.W.Y. Chan. School of Physical and Occupational Therapy, McGill University, Montreal, Canada, H3A 1Y5.

The correlation between the severity of spasticity and residual voluntary muscular activity is unclear, yet the latter is often used to investigate the effects of treatment in spastic movement disorders. The objectives of our study were to compare voluntary EMG and force generated by ankle plantar- and dorsiflexors in normal and spastic hemiparetic subjects, and to investigate the reproducibility of these measures and their correlation with clinical spasticity.

Seven age-matched normal subjects were tested once, and thirteen spastic hemiparetic subjects at least twice with each week apart. Subjects generated maximal isometric ankle plantar- and dorsiflexion in the standing position. Agonist and antagonist EMG areas, co-contraction ratios, maximal force and temporal characteristics of force production were compared between affected and non-affected legs of hemiparetic subjects, after the latter were found to behave similar to legs of normal subjects. Spasticity was evaluated by clinical scales.

In dorsiflexion, maximal agonist EMG area and force were significantly decreased to 39% and 35% respectively of the non-affected leg. During plantar- flexion, agonist EMG and force was reduced to 65% and 60% respectively of the non-affected leg. Measures of maximal and mean force, force onset and dorsiflexion co-contraction ratios were highly reproducible (r=0.78 to 0.99), while raw and normalized EMG area measures were less so. Most interestingly, the amount of dorsiflexing force produced by the paretic dorsiflexors was highly correlated with the amount of agonist/antagonist co-contraction (r=0.93), and inversely related to clinical measure of antagonist spasticity (r=0.65).

The high reproducibility of the force measurements suggested that they could be used to evaluate the effects of therapeutic interventions over time. More importantly, our findings demonstrated that, in hemiparetic subjects, the motor deficit in the paretic dorsiflexors but not the spastic plantarflexors was a reliable and valid indicator of the severity of spasticity.

S15.18


Following many types of CNS injuries, neurofunctional recovery gradually moves. Little is known, however, about the processes underlying this recovery. In spinal cord injury (SCI), the recovery time course is very consistent with regards to physiological and behavioral measurement. Recovery is determined for 1 wk, then gradually improves for another 2 to 3 weeks, and then plateaus.

To investigate the mechanisms of recovery after SCI, we developed a spinal cord injury device capable of measuring the precise impact velocity and extent of cord contusion. Three neurophysiological tests were used to assess function after SCI, which were graded: auditory brainstem responses (ABR), hindlimb myoelectric responses evoked by cerebellar stimulation (MEP), and somatosensory evoked potentials (SEP).

Rats were anesthetized and, using sterile surgical procedures, a T10 laminectomy performed to expose the spinal cord. The cord was contused and the sound closed. Evoked potentials were monitored before and after contusion. ABR, MEP, and SEP studies showed a similar time course to that found previously. Of considerable interest, however, was the fact that MEP amplitudes, which typically begin to drop between 3 and 0.2 Hz, showed a substantial increase in ability to follow higher frequencies which was maintained as long as 8 weeks after injury (see figure).

S17.1


Although several neuropeptides have been implicated in the pathophysiological and behavioral changes associated with Alzheimer's disease (AD), only corticotropin-releasing factor (CRF) and somatostatin (SRIF) have consistently been found to be reduced in post-mortem AD brains. The present study was designed to further evaluate these neuropeptides in 38 discrete brain regions. Tissue from 10 controls which consisted of both dememted (non-AD) and non-demented AD patients, were compared to tissue from 15 patients with neuropathologically confirmed AD. CRF and SRIF concentrations were determined by radioimmunoassay. CRF concentrations in AD patients were significantly lower in 11 cortical regions (Brodman's areas 4, 7, 10, 11, 12, 20, 21, 22, 39 and 44 and middle (posterior) gyrus) and the amygdala, insula, hypothalamus, and putamen. The concentration of SRIF was significantly decreased in 8 cortical regions (Brodman's areas 6, 17, 20, 21, 31, 32, 41, and 42). These results differ somewhat from previous studies in the pattern of neuropeptide alterations. Further investigation of the regional brain changes associated with AD may provide new insights into the pathophysiological processes.
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

S17.3
A LINE OF PLAQUES IN AREA 17 OF ALZHEIMER'S DISEASE: IMPLICATIONS FOR PATHOGENESIS. T.G. Beach and E.G. McGeer. Kinam Laboratory of Neurological Research, Univ. of British Columbia, Vancouver, B.C. Canada.

The laminar distribution of staining for senile plaques in area 17 was investigated in 16 patients with Alzheimer's disease (AD) and compared to the normal laminar distribution of affterent fibres, vascular plexus, and cholinergic nerve terminals, and other chemoarchitectonic features. Senile plaques were aggregated at significantly higher density at the interface of laminae IV and V. This did not correspond with any reported affterent, chemoarchitectonic, or vascular distribution. The line of plaques observed at the IV/V interface is similar to that in the dentate gyrus (Cain, B.J., et al, Acta Neuropathol., 74:87, 1988). The line of plaques in the dentate gyrus also has no apparent correlate in normal anatomy, but, as in area 17, is located at an interface between laminae. At both sites, the line of plaques form where an Air-50-immunoactive neuritic field (Hyman, B.T., et al, Brain Res., 450:392, 1988; Beach, T.G., et al, Brain Res., 501:171, 1989) marking degenerating neurites meets a layer which has a high capillary density and a high cholinergic terminal density. The interaction between one or more of these elements may therefore be contributive to senile plaque formation.

S17.5

The amygdala undergoes severe degeneration in Alzheimer's disease (AD). To assess for synaptic connectivity, we used a Golgi-Kopsch modification to study dendrites of the magnocellular basal amygdaloid nucleus (BNMC). The BNMC receives an especially dense projection from nucleus basalis of Meynert. Five cognitively normal aged controls were compared to 4 AD cases (postmortem ages 50-94; NINCDS-ADRD A-NIA criteria). Employing 15 neurons per case, the dendritic arbor was ordered somatofugally and each segment measured using an interactive, on-line computer tracing program (Kontron IPS). Mean total dendritic length per neuron was reduced in AD by 22%, due largely to changes among intermediate order segments. Significant alterations in total number of segments and average segment length, however, were not found. Despite the overall surface loss, increases in dendritic length and number with AD were noted amongst the most distal segments. These data suggest the presence of a subpopulation of excessively branched neurons in AD. However, since the majority have regressed dendritic trees, the net result is atrophy, suggesting a reduced amygdala connectivity with key CNS regions. Supported by Alzheimer's Association IIRG-87-042.

S17.6
UBIQUITIN IMMUNOREACTIVE DYSTROPHIC NERVIRES IN SELECT AREAS OF THE NEOCORTEX IN DOWN'S SYNDROME. L.A. Mattia, Y. Kosaka, P. Darwin, S.F. Henn and D.W. Dickson. Dept of Pathology, Albert Einstein College of Medicine, Bronx, NY, 10461. Ubiquitin immunoreactive structures were studied in Down's syndrome brains ranging in age from 2 days to 60 years. Numerous randomly distributed ubiquitin immunoreactive dystrophic neurites were seen in and around the cerebral cortex of these patients. The majority of these dystrophic neurites were detected in cortex and hippocampus of young (up to 12 years old) and middle aged (up to 60 years old) Down's syndrome patients. These dystrophic neurites were detected in cortex, hippocampus, and in the entorhinal cortex of young Down's syndrome cases. In the entorhinal cortex, myelin and neurofilament abnormalities were commonly seen. These abnormalities are similar to those previously described in Down's syndrome. The presence of dystrophic neurites suggests that these dystrophic neurites may be involved in the pathogenesis of Down's syndrome. This may provide a clue to the pathogenesis of Down's syndrome.

S17.7

Guam, one of the Mariana Islands in the Western Pacific, is characterized by a very high prevalence of ALS and Parkinson-Dementia (PD) in certain villages. We have undertaken a quantitative analysis of the laminar and regional distribution of neurofilamentary tangles (NFT) in the cerebral cortex of several cases presenting with different clinical symptomatology. Preliminary results revealed that areas within the temporal lobe were more heavily affected than the frontal or parietal regions in PD cases. In particular, the hippocampal formation contained the highest NFT densities (up to 200 NFT/mm cortical traverse). ALS cases had very few, if any, NFT. There were NFT that were more frequent in Guam case with Alzheimer's disease (AD) cases: NFT in neocortical association area of Guam cases always predominated in superficial layers (up to 3 times more NFT in layer V than in layer IV). NFT were more frequently observed in layer V. The motor cortex was consistently involved in Guam cases, while it has very few NFT in AD. No amyloid was observed in any of these Guam cases. The differential NFT distribution in Guam cases still supports the hypothesis that a global corticocortical connection occurs in dementia, but suggests that in these cases, there is a more marked reduction in layer V than in AD. These data are consistent with the notion that the disease may be caused by a pathogen retrogradely transported along specific corticocortical projecting axons, and may be more regional than in AD. Supported by grants from NIH AG06667 and AG08802.
S17.10
ENTORHINAL CORTEX IS AN EARLY SITE OF NEUROFIBRILLARY TANGLE PATHOLOGY IN NON-DEMENTED ELDERLY
It is known that the occurrence of Senile Plaques (SP) and Neurofibrillary tangles (NFT) seems to increase with age in nondemented elderly. We have studied the question of whether certain cytoarchitectural areas are consistently vulnerable, and whether there is a relationship between the appearance of NFT and SP on one hand, and age on the other. We examined 15 cytoarchitectural areas including the hippocampal formation, entorhinal cortex (EC), amygdala, nucleus basalis, and neocortical areas 20 and 21 in 20 cases of presumed non-demented elderly, using ThioflavinS (Thio S) and Alz-50 immunocytochemistry. Quantification of NFT (Thio S) showed that the greatest severity of involvement was in the EC. The severity of NFT pathology increased exponentially with age (R=0.60, p<0.05). The number of NFT in other areas (combined) was significantly correlated with the number of NFT present in the EC (Spearman correlation, p<0.04). Although amyloid SP were present in nearly all cases, Alz-50 positive SP and immunoreactive dystrophic neurites were found only in cases with substantial NFT (more than 35 NFT/8 mm² field) in EC. These results suggest that NFT and SP accumulate in a stereotyped, hierarchical fashion with age, and that the EC is an early site for NFT pathology. In addition, the presence of Alz-50 positive SP and dystrophic neurites is related to the severity of NFT. (Supported by the Brookdale Foundation, the Educational Commission for Foreign Medical Graduates and NIH AG00487). We thank P. Davies New York, for the gift of Alz-50.

S17.9
NEUROPATHOLOGIC CHANGES IN THE HIPPOCAMPAL FORMATION OF ALZHEIMER'S DISEASE PATIENTS: A QUANTITATIVE MRI ANALYSIS
Traditionally, the monitoring and diagnosis of a neurologic disorder using magnetic resonance imaging (MRI) was based solely on the visual images obtained. New low field (LF) MRI, however, greatly increase T1 and T2 tissue contrasts (which reflect the molecular environment of the tissue), allowing for easy and reliable quantification of different, or changing, tissues. Exploiting this advantage, T1 and T2 relaxation times of the hippocampal formation (HF) were measured in normals (age 18-79 yrs) and 4 patients with suspected Alzheimer's disease (AD)(age 69-81yrs) using an Instrumentarum Magnaview 0.4T LF MRI (T1 = 1R:1500 (5.375/40), T2 = SE:1000/130 and 1000/220). A mid-hippocampal, 10-mm coronal slice through the HF revealed T1 times of ADs to slightly, but consistently, exceed those of normals. T2 times of ADs, however, were substantially lengthened (by 15-65ms), and appear to be related to the degree of dementia. In addition, there were indications of differential pace of AD pathology in the two hemispheres. These results suggest that LF MRI may provide a means for diagnosing and quantifying the pathology of AD.

S18.1
TRANSFER KINDLING FROM THE AMYGDALA OR PIRIFORM CORTEX TO THE FRONTAL CORTEX IN FEMALE RATS WITH AND WITHOUT Estradiol REPLACEMENT. G. G. Raitzsch1, and G. M. Hudson. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.
In ovariectomized (OVX) female rats, estradiol (E) replacement facilitates kindling of the amygdala (Am) and frontal cortex (FCx), but not the piriform cortex (pCx). We therefore tested the influence of E on secondary ICx kindling following antecedent kindling of one or more ICx (Am or pCx) primary sites. Ten days after OVX, rats were kindled by daily Am or pCx stimulation. At least two weeks later, daily ICx kindling stimulations were initiated in these rats, with (E) and without (nE) estradiol replacement. Am-kindled nE rats completed secondary ICx kindling after 13.6±2.3 afterdischarges (AD) and 559±32 AD sec, and pCx-kindled nE rats completed ICx kindling after 13.5±24 ADs and 374±51 AD sec, significant savings compared to ICx primary site kindling of nE rats (38±2.2 ADs and 840±93 AD sec). In Am-kindled rats, E replacement did not significantly effect secondary ICx kindling. In pCx-kindled rats, E rats completed secondary ICx kindling after significantly fewer ADs (52%) and AD sec (44%), compared to nE rats. These results suggest that E facilitates the kindling process by interacting with an anatomical/mechanical substrate shared by E-sensitive kindling sites. (Supported by PHS NS20670).

S18.2
The perforant path demonstrates physiological and morphological plasticity including reversible increases in synaptic efficacy (LTP) and kindling-induced synaptic reorganization. CSD analysis was used to investigate functional consequences of these phenomena in dentate gyrus (DG). CSD analysis in normal rats revealed the location of currents underlying PP evoked field potentials in the DG. LTP increased the magnitude but did not alter the spatial pattern of PP evoked currents in the DG. In contrast, PP activation in kindled rats evoked an altered pattern of current flow including a net inward current in the inner molecular layer at 5-7 msec after granule cell discharge. The spatial location of this current corresponds to the terminal zone of sprouted mossy fibers, suggesting that axonal sprouting and synaptic reorganization of the mossy fiber pathway may have functional consequences that could include increases in recurrent excitation mediated by the sprouted collaterals.

S18.3
DEVELOPMENT OF POSTICTAL EEG DEPRESSION IN AMYGDALA-KINDLED RATS. B.M. Pedrick1, R.F. Serman, D. Psychology, Wayne State University, Detroit, MI 48202.
Seizures produced by the kindling process are usually followed by a period of flattened electroencephalographic (EEG) activity called postictal EEG depression. The development of this phenomenon was observed in treatment-free subjets. Twelve, male, Long Evans rats were stereotaxically implanted with electrodes into the medial amygdala. Electrical brain stimulation thresholds that produce a brief epileptiform EEG depression were established for each rat. Animals were stimulated at threshold until five consecutive Stage 5 or 6 behavioral seizures were attained. Observations were made of the development of the afterdischarge and the duration of postictal EEG depression. The development of postictal spiking was also recorded. Postictal EEG depression as evidenced by an "afterkindled" over repeated stimulation trials. However, correlations between the durations of afterdischarge and postictal EEG depression were uniformly low and not statistically significant (r=0.12) indicating that the underlying mechanisms responsible for these phenomena are different. Thus, kindling appears to be associated with long-lasting changes in both excitatory and inhibitory neural processes. (Supported by Grant No. R8-08167).

S18.4
AMYGDALA KINDLING INFLUENCES THE EXCITABILITY OF BRAINSTEM SUBSTRATES. C.D. Appelgate, G.M. Szaubik1, and L.J. Bur Refeld. Comprehensive Epilepsy Program, University of Rochester School of Medicine, Rochester, NY 14642.
Previous research has demonstrated a more epilepticogenic propensity for pontine reticular structures in the expression of tonic kindling extension (THE) following ECS stimulation (Browning, Fed. Proc., 44:2425, 1985). This study investigated the influence of amygdala kindling on THE. Sprague-Dawley rats were implanted with bipolar electrodes into the left amygdala (N=14). Following recovery, the incidence of THE to a corneal ECS-stimulus was determined. Rats were then kindled to 5 consecutive stage 5 seizures, and the incidence of THE was again determined. In comparison with unkindled control animals (N=23), kindling significantly increased the incidence of THE from 23% to 79%. The incidence of THE in controls was 36% and 39% for test and control, respectively. In a second experiment, amygdala-kindled rats (N=7) received mechanical lesions of the pontine reticular formation. While lesions blocked the expression of THE in 5/7 cases, no significant alteration in kindled seizure expression was observed. Results suggest that while kindling interacts with brainstem substrates necessary for aspects of tonic seizures, these same substrates are not necessary for kindled seizure expression.
518.5  

c-fos Expression in the Magnocellular Neuroendocrine System During Amygdala Kindling  
R.S. Greenwood, R.B. Meiler L. Riter* and J.N. Hayward, Dept. of  
Neurology and Psychiatry and Neurology Curriculum, University of North  
Carolina, Chapel Hill, NC 27590.

In previous studies we have shown that resting plasma vasopressin (VP) is elevated and the VP mRNA expression is further elevated in the magnocellular neuroendocrine system after amygdala kindling. Since c-fos expression is elevated in other areas of the nervous system after seizures and may be involved in expression of VP gene transcription measured c-fos changes during the early phases of amygdala kindling. A 48 mer oligonucleotide specific for c-fos was end labeled with [32P]dCTP and hybridized to RNA isolated from the brains of kindled or sham-stimulated rats. The hybridization of the antisense c-fos oligonucleotide probe was compared to hybridization of a sense oligonucleotide probe to a specific probe for VP mRNA under the same conditions as VP mRNA expression was measured. The fos mRNA in the cortex on the side of the amygdala changes was also higher than in sham stimulated animals.

The results of this study suggests that pro-genes could be involved in the regulation of vasopressin gene expression in kindled rats. Supported by NIH Javits Award NS 13411.

518.7  

THE EFFECT OF A 12-WEEK INTERVAL ON PERMANENCE OF 'PARTIAL' (STAGE 1), 'FULL' (STAGE 5) AND LOW FREQUENCY (3 Hz) KINDLING IN THE RAT.  
L. Dennison, S. National Research Council, C.P. Toronto, Ont., Canada  
S-62.

We have examined the effect of a 12-week interval on kindling to stage 3 or stage 5 and observed that both groups demonstrated equal permanence of kindling (S.N. Abbr. 1989, 310.9). Here we examined the permanence of partial (stage 1) 60 Hz kindling and full (stage 5) low frequency kindling. An amygdala rats and 4-electrode were kindled using 1-sec trains of 60 Hz pulses to stage 1 (ST1), 4 afterdischarges (ADs) or stage 5 using 3 Hz pulses (LF). A second control group had AD threshold only determined (ADT). Rekindling after 12 weeks was performed using 1-sec trains of 60 Hz trains in all rats. Results showed that ST1 and CONT required the same total ADs to kindle to stage 5; therefore ST1 demonstrated significant savings over the interval. LF and CONT required the same number of ADs to rekindle to the second stage 5; therefore LF kindling demonstrated similar permanence to 60 Hz kindling. Supported by an NSERC grant to DPC.

518.9  

SOMATIC AND INTERDICITAL BRAIN REGIONS INVOLVED IN HYBRIDIZED KINDLING IN THE GFP; FGF2 SENSITIVE AND SENSITIVE LOCALED KINDLING  
F.J. Fernandez-Mas, M.T.T. Matrin, M. D. Fernandez, D. V. M. Reiter  
Northwestern University, Chicago, IL 60611.

The pattern of the cortical propagation of amygdala afterdischarges (AWAD), was studied in daily kindled rats. By means of a computer program, 4-second samples of the recordings with an intracranial cortical tectum were recorded 1, 2, 14, 26 and 30 sec after the afterdischarges (AWAD), were studied during the process. Behavioral video tape recordings were simultaneous recordings. Power spectra (4-16 Hz) and power spectra of isolated AWAD were computed during each behavioral stage (1-5). The cortical projection of the afterdischarges activation throughout the process in different animals (1-5) was determined. The analysis of the cortical activity showed less spectral density than that of the AWAD and the projections corresponded to the prefrontal and frontal intercalated cortex, the inferior portion of the nucleus anterior amygdala, the anterior amygdala, and the nucleus anterior amygdala. The AWAD projection was more evident in the insular and anterior amygdala posterior cortices, bilaterally in the frontal lobe, the post-optic activation of the central temporal lobe appeared towards the end of the AWAD. The electrical and behavioral manifestations, e.g., the direction of circling, miosis, and the pre-  

518.10  


Unilateral electrical stimulation of a specific area in the inferior collicular cortex produces bilateral behavioral seizures, which progress from wild running seizures after acute stimulation to generalized seizures after repeated stimulation (McCown et al., Exp. Neurol. 91:257-267, 1984). Initially, we found that unilateral stimulation initiated non-synchronous, bilateral afterdischarge waves in the inferior collicular cortex that coincided with the behavioral seizure activity. However, unilateral kindling of the inferior collicular cortex did not influence the kindling rate from the contralateral inferior collicular cortex. Furthermore, cortical afterdischarge waves in the inferior collicular cortex did not alter ipsilateral seizure parameters. Using ['H]-2-deoxyglucose autoradiography, the unilateral nature of seizure activity in the inferior collicular cortex was substantiated. Thus, unilateral activity in the inferior collicular cortex is sufficient to cause bilateral seizure behaviors. (Supported by HD-03110 and NS-26595.)
OTOPHATIC CHANGES IN Dopaminergic pathways in the prefrontal cortex and nucleus accumbens after antiganglioside-kindling. P. Rada*, E. Morl* and L. Hernandez. Laboratorio de Fisiología and Universidad de Medica, Facultad de Medicina, Universidad de Los Andes, Merida, 5101-A, Venezuela

As antiganglioside-induced neuronal death and epilepsy called ‘forced normalization’ has been described. When anticonvulgent administration suppresses seizures, psychotic episodes appear, and vice versa, when antiepileptic administration antagonizes psychotic symptoms, seizures occur more often. It is believed that seizure and dopamine (DA) turnover are related. In fact, DA neuron blockers lower threshold seizures in rats. However, in brain homogenates, DA turnover changes have not been observed after antiganglioside kindling. Microdialysis was used to monitor extracellular DA and its metabolites in the nucleus accumbens (NAC) and the prefrontal cortex (PFC) is antiganglioside kindled rats. Under ketamine anesthesia, 10 male Wistar rats were chronically implanted with ipsilateral monopolar electrodes in the striatum for electrical stimulation; guide shafts for microdialysis were aimed to the NAC in 5 rats and to the PFC in the other 5 rats. After a week of recovery, animals were stimulated daily in the amygdala until three consecutive stage 5 motor seizures were elicited (square-wave pulses of 300 μA, 0.4 msec and 60 Hz). DA and its metabolites were assessed by HPLC with electrochemical detection. DA turnover decreased in the NAC and increased in the PFC with kindling. These opposite variations in extracellular DA as a result of kindling suggest dopaminergic mesolimbic and mesocortical involvement in epilepsy and a mechanism for the ‘forced normalization’ phenomenon.


S1.11

EPILEPSY: INFLUENCE OF ANTI-ALZHEIMER'S ANTIBODIES ON AMYGDALOID MANIFESTATIONS IN THE RAT. K. A. Giordone, B. Coleman, and J. W. Goethe. Dept. of Biochemistry, University of Florida, Jacksonville, FL 32210, USA

Intracerebroventricular (ICV) injection of anti-HLA-DR antibodies into the lateral ventricles of rats with established Alzheimer's disease (AD) was shown to decrease the accumulation of amyloidotic plaques and neurofibrillary tangles. The effect of these antibodies was dose dependent and lasted for at least 5 days after injection. The results suggest that anti-HLA-DR antibodies may be useful in the treatment of AD.


3Blackwood, D. In P. L. Morselli et al. (Eds.), Raven Press: NY, 1981.

ALZHEIMER'S DISEASE: MOLECULAR STUDIES

S1.1


pADHC-9 is a hippocampal cDNA clone which is overexpressed in Alzheimer's disease hippocampus (May et al., 1989, Can J Neurol Sci. 16:473). Sulfated glycoprotein-2 (SGP-2), the rodent homologue of pADHC-9, is a major secretory product of the rat Sertoli cell and presumably is involved in male reproductive function; its role in brain is unknown. To explore possible functions, young male F344 rats were subjected to bilateral intraventricular injections of kainic acid (0.5 μg/ul each) and sacrificed 14 d later. Total RNA or protein was extracted from the dorsal hippocampus and in RNA or immunoblot analyses, SGP-2 RNA levels increased 2 fold in KA-lesioned hippocampi compared to saline-injected controls (p<0.05; n=4). As expected, GABA receptor expression in hippocampus also increased 4 fold following KA lesioning (p<0.02; n=4). Immunoblot analyses of hippocampal homogenates revealed comparable increases in SGP-2 and GABAA protein expression following the lesion. Immunocytochemical analyses detected little SGP-2 immunoreactivity in non-lesioned animals but marked accumulation of SGP-2 in atrophic CA3 and CA4 pyramidal cells present 14 d after the lesion. These data indicate that the increased expression of pADHC-9 in AD hippocampus can be duplicated in the kainate-lesioned rat and this model will be useful for identifying the function of pADHC-9/SGP-2 in the brain.

S1.3

INTERLEUKIN-1 (IL-1), INTERLEUKIN-6 (IL-6) AND TUMOR NECROSIS FACTOR (TNF) IMMUNOREACTIVITIES ARE EXPRESSED IN MICROGLIA IN HUMAN BRAIN. D Dickson, L Mitalic*, S-H Yang* and P Davies. Dept of Pathology (Neuropathology), Albert Einstein College of Medicine, Bronx, NY.

We have developed immunocytochemical methods for microglia in human brain to study the role of microglia in amyloidogenesis in aging and Alzheimer's disease. Microglia share a number of antigenic properties with macrophages, including presence of epitopes to class II major histocompatibility antigen (HLA-DR), Leu-M5 and leukocyte common antigen (LCA). Microglia also produce a number of cytokines, including IL-1, IL-6 and TNF. In tissue fixed briefly in picolade-containing paraformaldehyde and sectioned with a Vibratome, microglia were immunoreactive with antibodies to IL-1, IL-6 and TNF. In Alzheimer's brains, microglia were distributed in a reticular array in gray and white matter, and were clustered in areas of amyloid deposition. In control brains from AD patients, microglia were immunostained. Double labeling studies showed that cytokine-reactive cells were microglia and not astrocytes co-labeled with antibodies to HLA-DR, Leu-M5 and LCA, but not antibodies to glial fibrillary acidic protein.

S1.4

INTERLEUKIN-18 mRNA LEVELS INCREASE IN ASSOCIATION CORTEX IN ALZHEIMER'S DISEASE. K. H. Rogers, A. B. Wadhams*, P. D. Coleman. Dept. of Neurobiology and Anatomy, University of Rochester, Rochester, N.Y. 14642, USA

Interleukin-18 (IL-18) is a cytokine which has been shown to stimulate astrocyte proliferation. Additionally, it can induce expression of the B-amyloid precursor gene. Thus, we have examined IL-18 mRNA levels in superior frontal gyrus in normal aging and in Alzheimer's Disease (AD). Batch mRNA isolations were performed from 24 AD and nine age-matched control cases. Integrity of the mRNA was measured by Northern gel analysis and mRNA content was quantified by hybridization to an oligo probe. Serial dilutions of each sample were bound to a nylon membrane and probed with a cRNA transcript of human IL-18. Resulting autoradiograms were quantified by scanning densitometry. The total yield of mRNA per gram of tissue did not vary significantly between normal controls and AD cases. In control cases less than 80 years of age, IL-18 transcripts were not detected. IL-18 message was present in control cases 90 years and older, although at relatively low levels. In AD cases IL-18 messages were present in all age groups. However, the IL-18 message levels were higher in young AD cases than in AD cases 90 years and older. Thus, IL-18 mRNA shows an age-related increase in normal aging and an age-related decrease in AD. (Supported by AG 09016, AG 001721, AG 03644, AG 00107, PKG 89-120)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
519.5 ANOMALOUS REACTION OF OLIGO PROBES FOR 3 HEAT SHOCK mRNAS IN ALZHEIMER HIPPOCAMPUS. M. Morrison-Bogorad, S. Parrish, K. Groshen, C. White, I. B. Bonzo, B. Border, E. K. Miller, R. Gonzales and J. D. Reisine. Dept. of Neurology, University of Massachusetts Medical Center, and Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX 75235, and The Schizophrenia Research Center, Veterans Administration Medical Center, Dallas, TX 75216.

The stress protein, ubiquitin, is expressed in some affected neurons in Alzheimer disease (AD). Little is known about the expression of other members of the heat shock family in AD. We hybridized oligo probes specific for human hsp70, hsp70 and hsc70 mRNAs to cerebellum and hippocampus from control and AD brain sections. Sections were counterstained with congo red to demonstrate AD pathology. 32P-labeled probes showed a strong signal highest for the hsc70 probe, in CA1-2, subiculum and entorhinal cortex of 6/7 AD hippocampi. These regions contained plaques and tangles. Grains were present in pyramidal neurons and were often associated with neurofibrillary tangles. No comparable reaction was present in either region of control brain or in AD cerebellum. Post-hybridization washing at high stringency (0.1 X SSC, 45°C) reduced but did not abolish signal. In 6/7 cases, signal intensity was markedly increased when sections were pretreated with ribonuclease. In contrast, a control 18S rRNA oligo showed normal cell-specific hybridization to each brain that was always completely eliminated by ribonuclease pretreatment. Further experiments will determine whether the anomalous reaction with the heat shock probes in affected regions of AD hippocampus results from an increase in any mRNA specificity. Supported by NIH grant AG16013 and by the Leland Fikes Foundation.

519.6 A DEVELOPMENTALLY EXPRESSED PROTEIN ISOLATED FROM NORMAL RAT NEOCORTEX. M. Marcraugh, R. Maroko, J. Ebbli, and N. Miller. Dept. of Anatomy, Univ. of Md. and Dent. B.J. Sch. of Osteopathy, Sunbury, PA 18940, and Dept. of Biochemistry, University of California, Davis, CA 95616.

We isolated a protein from the cerebral cortices of patients with Alzheimer's Disease and Down's Syndrome and from normal human fibroblasts. An 85-kDa protein was isolated from the cortices of normal human adults. A recent immunohistochemical study has shown that 85-kDa also identifies a population of cortical cytoplasmic neurons. We isolated the antigen recognized by 85-kDa in rat cortex using immunofinity column chromatography; the column was bound with ALZ-50 and eluted with 10 M potassium isothio-cyanate. Unlike the Alzheimer's antigen (M.W. 68 kDa), the isolated rat protein had a molecular weight of about 50 kDa. This antigen was evident during the first postnatal week and its expression waned during the second postnatal week. Both the molecular weight of the neuronal antigen and the timing of its appearance are reminiscent of rat juvenile tau. Funded by DE 07734 and AA 06916.

519.7 ANALYSIS OF GENE EXPRESSION IN ALZHEIMER'S DISEASE USING THE POLYMERASE CHAIN REACTION. T. Golde, M. Cohen, T. Cheung, S. Estus, C. Hopter, B. Kalani, L. Youklin, and S. Youklin. Case Western Reserve University, Cleveland, OH 44106.

To analyze the role of altered gene expression in the evolution of AD pathology, it is essential that methods be developed to analyze multiple mRNAs in the samples that can be obtained from discrete brain regions wherein pathology is well defined. In a previous study, we analyzed alternatively spliced 8 amino acid protein precursor to cell adhesion molecule by using the polymerase chain reaction to amplify BAPP mRNAs produced by reverse transcription. Since this method appears to be well suited for the analysis of gene expression in AD, we have synthesized oligonucleotide primer pairs specific for BAPP, B-actin, cyclophilin, GFAP, alpha-1-antichymotrypsin, serum amyloid P, IL-1b, MARCKS, and c-rat mRNAs. Using these primer pairs, we amplified cDNAs specific for each mRNA from 1 μg or less of randomly primed reverse transcribed total RNA from AD or control brain, and we have confirmed by direct sequencing that these cDNAs do, in fact, correspond to the mRNAs targeted. Optimization of the amplification process has enabled us to simultaneously amplify up to 6 different cDNAs from 1 μg of RNA with minimal amplification of non-specific products. Moreover, by diluting randomly primed white matter cDNA, we find that the relative amounts of up to 5 different messages can be quantitated in a single PCR reaction as long as amplification is in the exponential phase and the messages are roughly equal in abundance. Furthermore, by comparing the relative amounts of 67 bp and 300 bp 9-actin cDNAs produced with appropriate primers, it is possible to evaluate the extent of degradation present in each postmortem RNA sample and to select, on the basis of the 67 bp / 300 bp ratio, samples in which degradation has minimal effect on the level of the mRNAs measured. We are currently using this method to analyze expression of many mRNAs in various regions of AD and control brains.

519.8 EPIDERMAL GROWTH FACTOR RECEPTOR EXPRESSION IN BRAIN, PITUITARY AND SKIN OF DEMENTED AND NONDEMENTED ELDERLY PATIENTS. L. E. Groshen, M. Millar, W. H. Clevin, and J. Rogers. L. J. Roberts Center, University of Nebraska Medical Center, Omaha, NE 68198.

The epidermal growth factor receptor (EGFR) is a 170KD integral membrane protein, which can bind to the extracellular domain of the EGFR and is capable of phosphorylating a variety of cellular proteins related to cell growth. Increased EGFR expression in brain, skin or other tissues may be accomplished by the binding of Vaccinia virus growth factor and transforming growth factor-α (TGF-α) to the extracellular domain of the EGFR. We have previously reported the presence of EGFR in the neonatal brain by immunoperoxidase staining. In the present study, we evaluated the distribution of pigmented (n=7), albinoid (n=1), and albino (n=1) control and demented patients (Alzheimer's, Parkinson's, multi-infarct, and Pick's disease) brains. We have shown that vascular EGFR immunoreactivity in the pituitary is strongly correlated with dementia. Three of three demented patients exhibited strong pituitary vascular EGFR immunoreactivity, whereas 3 of 6 controls lacked EGFR expression. The net anomalous control patients were predominantly high neurite plasma and tangle pathology. EGFR immunoreactivity was virtually undetectable in the pituitary of Alzheimer's patients, and was reduced in Pick's disease patients. These findings indicate that in demented patients the CNS may be a source of an EGFR inducing substance, or that vascular endothelial cells may have differential sensitivity to the inducing substance depending on their systemic location.

Supported by NIA AG07367 to JR.


We have shown previously that polysomes isolated from AD brains can be directly translated in vitro in the presence of a 32P-labeled amino acid. Ribosomes were purified from control and AD polysomes and characterized to determine the mechanism responsible for this disruption. Control and AD ribosomes equally supported translation of hemoglobin mRNA in the in vitro translation assay (69.2 ±17 vs. 76.4 ±44 X 10⁶ dpm 35S-met) [A260 unit. n = 5]. (Liewehr, the physical integrity of ribosomal RNA, appeared similar by northern analysis of 18S rRNA. However, examination of ribosomal proteins by two dimension gels indicated that the polysomes that were present in the AD ribosomes (50 Kd, 7.8; 30 Kd, 7.8; and 16 Kd, 8.2). These differences appear to be due to alterations in the pattern of ribosomal proteins. Such alterations may represent differences in post-translational modifications of the proteins, such as protein phosphorylation. The altered proteins are currently being identified and their phosphate content, if any, measured.


Nuclear run-on analyses give in vitro measurements of relative transcription rates for individual genes. As shown in other tissues, nuclear run-on can be used to monitor rapid transcriptional changes in response to various experimental stimuli. To study the effects of Alzheimer's disease on gene expression, we developed a nuclear run-on transcription assay using nuclei isolated from frozen brain tissue. Initial studies showed no difference in yield of nuclei per tissue mass, or amount of run-on transcript produced, between nuclei from fresh or frozen rat brains. Transcription was dependent upon added ribonucleotide triphosphates. Freezer storage time had no apparent effect upon the yield of nuclei or labeled run-on transcript produced. Nuclei isolated from frozen human post-mortem brain produced equivalent amounts of run-on transcript per isolated nuclei, as compared to fresh brain tissue nuclei. Brain cell nuclei can be separated by sucrose gradient centrifugation into discrete sub-populations: large (n=3), and small (n=4) nuclei. Large nuclear nuclei are thought to be of neuronal origin, while the fraction containing small nuclei represents glia and small neurons (Sarkander and Uthoff, PFEBS Lett. 15, 137). Therefore, a new approach to labeled run-on transcription to cDNAs for b-tubulin (neuronal and glial) and tyrosine hydroxylase (neuronal), but not for GFAP (astrocyte). The small nuclear transcription showed hybridization to GFAP cDNA and allowed discrimination of neuronal and glial transcription rates in human brain. Supported by NIA AG07908 to C.E. Finch.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
THE PROTEIN ENCODED BY pADHC9/SGP2 IS EXPRESSED IN ALZHEIMER'S DISEASE HIPPOCAMPUS AND IS ELEVATED IN THE RAT HIPPOCAMPUS FOLLOWING ENTORHINAL CORTEX LESION.

M. Lempert-Etchells, F. A. McMorris, T. Engel, C. E. Finch, Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089

Clone pADHC9 encodes a 2 kb RNA which is overexpressed in Alzheimer's disease (AD) hippocampus and is the human counterpart to rat tetratolic SGP2. (JIRG 1989) PNAS 86, 7123. Polycistronic antisense was made to the human pADHC9/SGP2 protein to study the level of expression, the cellular distribution, and the function of this protein in Alzheimers hippocampus. An XhoI fragment of pADHC9/SGP2 from nucleotide 921-1500 was inserted into the E.cell expression vector pATH1. The fusion protein made from this vector was used to generate rabbit antiserum to pADHC9/SGP2 protein. Western blot analysis with the IgG fraction identified the expected 40kD reduced protein in human serum and hippocampi of similar 40kD protein in human spinal fluid as in human Alzheimer and control hippocampus extracts this antisense identified the 38kD reduced form of this protein along with its 55kD precursor. The polyclonal antisera was used to compare different types of glycoprotein bands.

In the rat, entorhinal cortex lesion was used as a model for aspects of the hippocampal degeneration seen in AD. By northern blot analysis SGP2 mRNA was elevated in the hippocampus several fold by 2 days post lesion. It reached a peak at 6 days and decreased to normal levels by 14 days post lesion. The SGP2 protein at 4 days post lesion was also increased relative to the uninjured contralateral hippocampus. We conclude that SGP2 mRNA and protein respond early to brain lesions and are targets for a new probe for molecular changes in AD. Supported by ADRDA JIRG-B8-069 and NIH/NIA AG07909.

FRIDAY AM SYMPOSIUM

522 SYMPOSIUM: PHYSIOLOGY OF PEPTIDERIC NERVE TERMINALS IN THE VERTEBRATE NEUROHYPOPHYSIS.

C. W. Bouma

The hypothalano-neurohypophysial system of vertebrates consists of the hypothalamic somata of magnocellular neurons synthesizing either oxytocin or vasopressin and their axonal projection to the posterior pituitary. Because of their relatively large size (several μm) and compartmentalization, neurohypophysial terminals have become a classic preparation to examine the physiology of peptide secreting terminals. This symposium will highlight a number of convergent advances in understanding the mechanisms that modulate the coupling between axonal firing to nerve terminal excitation and secretion from this neuroendocrine system. Russell will review the effects of activity patterns on neuroepitope secretion from the neurohypophysis, and describe the modulation of intrasynaptic (Ca2+) signals in isolated terminals. Obaid will relate the activity-dependence of spike invasion and excitation of nerve terminals to the effects of firing patterns on peptide secretion. The mechanisms of Ca2+ influx in the intact terminals of the neurohypophysis as probed with potentiometric dyes will be described by Obaid. Saltzberg will describe changes in light scattering consequent to nerve terminal excitation and detail their relation to excitation-secretion coupling. Nowacky will describe patch clamp studies of neurohypophysial Ca2+ channels in isolated terminals and discuss their role in exocytosis as studied with capacitance measurement techniques. Finally, Lemos will characterize ion channels in isolated terminals and neuropeptorinary granules, and comment on their possible role in the control of peptide secretion.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

523.1 INTER-AREAL AND INTER-HEMISPHERIC SYNCHRONIZATION OF OSCILLATORY RESPONSES IN CAT VISUAL CORTEX.


We have previously demonstrated that oscillatory responses can synchronize across orientation columns in area 17 of cat visual cortex. We proposed that this synchronization reflects a temporal coding mechanism for scene segmentation. This hypothesis predicts that 1. similar oscillations should occur in other visual areas, 2. there should be evidence for inter-areal synchronization and 3. inter-hemispheric synchronization should occur. To test these predictions, we made simultaneous recordings of multi-unit activity from area 17 and PMLS in 4 adult cats. In 3 additional cats, we recorded simultaneously from area 17 of either hemisphere. The oscillatory nature of the responses and the strength of synchronization were quantified by computation of auto- and cross-correlograms. We obtained the following results: 1. More than 70% of the PMLS recordings displayed an oscillatory modulation in the frequency range of 40-60Hz. 2. In more than half of the cases tested, we observed a response synchronization between area 17 and PMLS. 3. The inter-areal response synchronization depends on the overlap of the receptive fields, but not on differences in orientation preference. 4. The inter-PMLS interaction is sensitive to global stimulus features, such as contour of contours and coherence of motion. 5. In 70% of the cases we recorded simultaneously from area 17 of both hemispheres, we observed a response synchronization. The interactions are as strong as those observed in similar recording strategies in cat visual cortex and that response synchronization can involve cells located in different hemispheres or visual areas. The inter-hemispheric synchronization may serve for the binding of different feature maps. The inter-hemispheric interactions may provide a mechanism to link features of objects extending across the vertical meridian.

523.2 SQUINT AFFECTS OCCURRENCE AND SYNCHRONIZATION OF OSCILLATORY RESPONSES IN CAT VISUAL CORTEX.

Peter König*, Andreas K. Engel*, Sigrid Lütolf* and Wolf Singer Max-Planck-Institut für Hirnforschung, D-6900 Frankfurt 71, FRG.

We could recently demonstrate that neurons in cat visual cortex display responses with an oscillatory temporal structure in the range of 40-60Hz which can synchronize over distances of 7mm in area 17. This synchronization may serve to establish relations between features in different parts of the visual field. We have now investigated occurrence and synchronization of oscillatory responses in area 17 of strabismic cats. We wondered whether in these animals response synchronization primarily occurs between cells of similar ocular dominance (OD). We recorded multi-unit activity (MUA) to appropriately oriented moving light bars simultaneously from 4-6 spatially separate sites in cortical area 17. We used 4 adult cats in which divergent strabismus had been surgically induced at the age of 3 weeks. To determine the temporal relationship of the firing patterns, we computed auto- and cross-correlation functions of the spike trains. Analysis of 112 recording sites gave the following results: 1) 87% of the responses were monocular or strongly dominated by one eye. 2) The occurrence of oscillatory response was with 65% as frequent as in normal cats, but response rhythmicity was much weaker. 3) Only 30% of the 115 pairs of MUA recordings showed a response synchronization versus 60% for overlapping receptive fields in normal cats.

Our data support the hypothesis that the functional organisation of area 17 is profoundly altered in strabismic cats. The weak oscillatory activity might reflect disturbed integrative capacities in the visual cortex of squinting animals. The preferential synchronization between neurons driven by the same eye suggests that cells with different OD become functionally independent, which implies not only changes in thalamo-cortical but also in cortico-cortical connectivity.
523.3 Temporal Dynamics of Oscillatory Neuronal Interactions in Cat Visual Cortex. Charles M. Gray, Andreas K. Engüle, Peter Koene* and Wolf Singer, Max-Planck-Institute for Brain Research, 6000 Frankfurt/M 71, F.R.G.

Previously we have demonstrated that a subpopulation of visual cortical neurons exhibit oscillatory responses to their preferred stimuli at a frequency near 50 Hz (Gray and Singer,PNAS,1989). These responses can selectively synchronize to the spatial or to the temporal features of visual stimuli (Gray et al.,Nature, 1989). Here we report the results of a new analysis which reveals the fine temporal structure inherent in these interactions. We utilized pairs of recordings of local field potential (LFP) activity from area 17 in the cat which met two criteria: The LFP was correlated with the underlying unit activity at each site and the recording sites were at least 3 mm apart in cortex. A moving time-locked correlogram was applied to the time-lagged cross-correlograms on 100 mseu epochs of data repeated at intervals of 30 mseu for a period of 3 sec during each direction of stimulus movement. And a statistical test was devised to determine the significance of observed temporal correlograms. In this way we were able to determine the magnitude, phase-lag, frequency and duration of correlation events as well as an estimate of the time needed to achieve phase-locking. The results demonstrate that 1) synchrony can be established within 1-2 cycles of oscillation, 2) the duration of synchrony is variable and lasts from 50-500 msec, 3) the phase and frequency of synchronized events is variable within and between regions or neurons + - 3 msec and 40-60 Hz, respectively, 4) multiple correlation events can occur within a single stimulus period. These results demonstrate a high degree of dynamic variability of neuronal interactions which is consistent with the requirements of a mechanism for feature integration.


We have examined the effect of texture patterns moving outside the classical receptive field (CRF) on the spatial sensitivity profile of single units in striate cortex (VI) of anesthetized macaque monkeys. Our stimuli consisted of a target and a moving background. The target was an optimally oriented line element flashed at different locations along an axis orthogonal to the preferred orientation within the CRF, and in different phases relative to the background motion. The background was a random dot pattern moving entirely outside the CRF, and oscillating at a temporal frequency of 2Hz along this axis. Responses were compared for 3 to 5 positions of the flashed target, and across 4 phases of the background motion.

In a minority of cells there was a differential effect, such that targets presented at a particular phase were enhanced at one target location and suppressed at another. These observations suggest that complex spatio-temporal interactions between target and background occur in VI.

523.7 SINGLE UNIT AND 2-DEOXYGLUCOSE STUDIES OF MULTI-CYCLE INHIBITION IN MACAQUE STRIATE CORTEX: R.T. Bora and R.H. Tootell, Dept. of Neurobiol., Harvard Medical School, Boston, MA.

In the course of single unit studies to map spatial frequency tuning in supragranular striate cortex of the macaque monkey, we discovered that a large population of neurons in the interblob regions responded poorly or not at all to extended gratings, but gave vigorous responses to single bars or edges. To characterize this property we have recorded from 93 single units in the interblob of layers 2 and 3 in striate cortex of the anesthetized, paralyzed macaque.

In those cells that became effective stimuli only when they were spatially delimited from the sides so that the grating had fewer cycles. This property, which we call multi-cycle inhibition, was present in 65 (70%) of the neurons we studied. It was independent of frequency and phase (28%). Thirteen of these cells studied quantitatively gave a maximal response to 1.3 /- 0.6 (mean +/- s.d) grating cycles and a maximal response to 3.5 /- 1.7 grating cycles. In a few cells tested with multiple cycles, the inhibition pattern occurred only when the orientation of the surrounding grating was similar to that of the center. In 2 animals, 2-deoxyglucose was used to compare brain activation produced by a sine-wave grating while a bar width but 60% of the mean grating was present. In the other half of the cells, the orientation was equal (5 and 6) or greater for the sine-wave grating (4C). Subsequent single units studies have shown that the 2-deoxyglucose result is not due to low-pass modulation of the interblob.

Multi-cycle inhibition is a short-range spatial antagonism between contrasts of like orientation. We think of these cells as 'contour-pass filters' that may help to discriminate high contrast, texture-like regions of a visual scene from contours representing object boundaries. Supported by grants NIMH 14275 and EY 09780.

523.4 ORIGINS OF OSCILLATORY ACTIVITY IN THE CAT'S VISUAL CORTEX. G.M. Ovass, R.D. Freeman, Biophysics and Neurobiology Groups, Univ. of Calif., Berkeley, CA 94720.

The activity of neurons in the visual cortex, generally studied by spiking activity or gross potentials, has been reported to exhibit oscillatory firing patterns. We have analyzed spike trains of cortical neurons and lateral geniculate fibers in the visual cortex of the cat under different conditions of visual stimuli, viz. texture, or their nature and origin of these oscillations.

Two types of visual stimuli were presented for extended periods of time to determine the stability and spatial dependence of oscillatory firing. The first type consists of large drifting gratings of sinusoidal intensity at optimal orientations and spatial frequencies. The second type of stimuli consists of random sequences of small optimally oriented bright or dark bars flashed for 50 ms at different locations within and around the receptive fields. For both stimuli power spectra of extracellularly recorded action potentials are computed to provide qualitative assessment of the degree of oscillatory firing. Oscillatory firing, as exhibited by a peak above noise at around 50 Hz in the power spectrum, is observed in 39% of the cortical cells and LGN fibers studied by grating stimulation. The oscillations of several LGN fibers are among the strongest found. Oscillations are generally highly variable over a period of seconds in both frequency and magnitude. Moreover, the relative magnitude of this oscillatory firing is sometimes inversely related to firing rate. These findings suggest that such oscillations are at least in part due to intrinsic spontaneous firing properties. Oscillations do not depend on the presence of large coherent stimuli, since 39% of the cells studied with small bars also fire synchronously at 50 Hz. In conjunction with previous studies in the LGN and retina, these results support the notion of a subcortical, rather than intracortical, origin of oscillatory firing. (EV01175)

523.6 SPATIAL ORGANIZATION OF SUPPRESSIVE SURROUND EFFECTS IN NEURONS OF AREA V1 IN ALERT MACAQUE. L. J. Krinerson and D. C. Van Essen, Caltech, Division of Biology 216-76, Pasadena, CA 91125.

In a majority of neurons in area V1, responses to a line segment within the classical receptive field (CRF) are suppressed by a texture pattern lying entirely outside the CRF. We studied the spatial organization of these texture suppressive effects using small patches of drifting sinusoidal contrast, since we have previously shown that orientation contrast reduces the suppressive effect in many cells (Krinerson & Van Essen, Soc. Neuron. Abstr., 1985). For the sample of 122 cells tested, both the flank squares and the end quadrants suppressed the response by an average of 25%, which was smaller than the 35% suppression induced by the full surround. Thus, the suppression effects are spatially distributed and not exclusively attributable to end-stopping or to side-band suppression. In each case, the suppression induced by the full surround was about 20% greater than that induced by the orientation contrast surround. However, we did not confirm a spatial asymmetry for the subset of 39 cells that showed a significantly greater response to orientation contrast than to uniform orientation on the full-field test. In these cells, for the end quadrants, the uniform orientation surround suppressed the response 17% more than the orientation contrast surround whereas for the flank quadrants, the difference in suppression was 9%. Thus, the orientation contrast effect originates from both the end zones and the flanks, but more strongly from the former.

We also recorded responses to a full-field texture in which the surrounding line segments were randomly oriented. This stimulus is similar to the uniform orientation surround in that there is no orientation contrast between the line segment in the CRF and the flanks. Of the 50 cells tested, 9 responded more strongly to the orientation contrast than to the random orientation texture, whereas only 3 responded in the opposite fashion. These differences were comparable to those for the uniform orientation comparison. Thus, the differential effects depend on the overall presence or lack of orientation contrast, rather than on the presence of a uniform orientation surround.

Intrinsically long-distance interactions and feedback inputs from higher cortical areas are two mechanisms proposed to account for some of the receptive field properties seen in primary visual cortex (area 17). We have examined whether inhibitory neurons participate in such circuits by combining retrograde labeling and immunocytochemistry with a monoclonal antibody to glutamic acid decarboxylase (Chang and Gottlieb, J. Neurosci. 8123, 1988).

Following injection of fluorescent latex microspheres into area 17, double labeled GABAAergic neurons can be identified in flat mount sections several millimeters lateral to the injection site in the extrastriate visual area 18a. In parasagittal sections through area 17, local inhibitory neurons are seen in layers 1-6 within 0.3mm of the injection tract. However, a second population of double labeled cells with much more extensive collateral arbors are seen at the layer 5/6 border up to 1mm from the injection site.

Occasionally, cells with a similar distribution are also encountered in layer 2/3.

These results suggest that neurons in primary visual cortex are under direct inhibitory feedback control from specific higher visual areas. They also suggest that direct, inhibitory, long-range connections do exist in rat primary visual cortex and that they are mediated by GABAergic neurons with a unique laminar distribution.

Supported by NIH EY05935.


Amino acid mediated excitatory neurotransmission plays an important role in the plasticity of intracortical circuits in developing and adult animals. To identify the cells which participate in these processes and to understand the organization of these axon collaterals, we have used retrograde tracing with D-/-H-aposilin to label primary visual cortex.

Injections into superficial and middle layers labeled cells in layers 3-6 in a stereotaxic horizontal and sublamellar distribution pattern. The projections within layer 3 and from the top and the bottom of layer 5 were more discrete than from layer 4 and 6 which were contained within a 0.5mm wide column. Typically, labeling is layer 2 and the corticocortical output zone in the middle of layer 3 and sparse. Alternatively, injection into deep layers produced a different labeling pattern and revealed long-range connections (-10mm) within the bottom of layer 5 and the top of layer 6. The projections from layers 3-4 into the sublaminar only in the lower half of layer 6 were narrow (<0.5mm) and topographically precise.

Interestingly, cells at the gray/white matter border in layer 6 which are known to project widely throughout all layers were always confined to a small region below the injection site. These results suggest that we have selectively labeled glutamatergic/aspaertic neurons and that they participate in specific circuits which mediate short and long-range interactions.

Supported by NIH grant EY05935.


Previous studies led us to hypothesize that retinal precursor cells remain plastic after terminal mitosis. Unless induced to develop as neurons by intraretinal signals, cells will follow a photoreceptor "default pathway" (Sclienze, 245:391-393, 1989). This hypothesis predicts that precursors that undergo terminal mitosis in vitro, and therefore differentiate in the absence of intraretinal signals, should give rise to photoreceptors at all stages. We tested this prediction by in vitro labeling of dividing precursor cells dissociated from embryonic day (E0) retina, using NS22789 (BNS8918951) and BNS8918951. Their fate was determined by phase contrast microscopy, opsin immunocytochemistry and sequential photomicrographs revealing increases in size, number and length of the first day in vitro, with cells becoming phototactic on the second day in vitro. In all cases, 80-95% of the differentiating cells showed the photoreceptor phenotype, regardless of the phenotypes of neighboring cells that were already phototactic at the time of their culture. These observations are consistent with and add support to the photoreceptor default pathway hypothesis.

324.2 A RETINAL ANTIGEN WITH ALTERED STAINING PATTERN DURING METAMORPHOSIS IS ALSO EXPRESSED BY NEURAL CREST, SPERMATOGENIC, AND G. Kirchbaumer.6 Department of Biology, City College of New York, NY, 10031.

Using immunosuppression methods, we generated IP56, an antibody whose expression appears to be developmentally modulated during both metamorphosis and embryogenesis in X. laevis. In premetamorphic retinas, IP56 stains pigmented epithelial cells and the outer segments of photoreceptors. During metamorphosis, staining is lost from retinal, becoming restricted to the epithelium and the peripheral stem cells. The change in staining pattern correlates temporally with metamorphosis, but appears to be independent of thyroid hormone. Blocking metamorphosis does not prevent the loss of immunoactivity in central retina, nor does intraretinal injection of thyroxine accelerate the change in staining pattern.

Although IP56 was raised against retinas from young metamorphic frogs, it also stains the eye rudiment, ectoderm overlying the eye, and neural crest of embryos. Both neural crest and the retinal germinal zone are stem cell populations, and each individual precursor may give rise to pigment cells as well as to neurons. Work in progress is aimed at determining why immunoactivity is lost from central retina, and the significance of co-expression by precursor cells of both the central and peripheral nervous system.

Supported by NSF BNS 8616730 and NIH R29 NS25042.
SOCIETY FOR NEUROSCIENCE ABSTRACTS. VOLUME 16, 1990
52.4.10 ROLE OF BRAIN-DERIVED NEUROTROPIC FACTOR (BDNF) AND GROWTH FACTOR (NGF) IN THE COMMITMENT OF PLURIPOTENT NEURAL CREST CELLS. M. Seber-Stoll. Dept. of Anatomy and Cellular Biology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226.

The influence of BDNF and NGF on the expression of traits specific for sensory and autonomic neurons by neural crest cells was examined. Cells that were not committed to an autonomic or sensory fate give rise to melanocytes, adrenergic, sensory neurons, and to its yet unidentified cells. The mechanisms that lead to these phenotypic restrictions are largely unknown. Neural crest cells at dorsal widths were grown in the presence or absence of BDNF (10 ng/ml) and NGF (25 ng/ml) on a collagen-laminin-fibronectin substrate. All cells within a colony were analyzed by triple labeling, using antibodies against the stage-specific embryonic antigen 1 (NFP), that identify in the cells sensory neuron lineage and against dopamine-β-hydroxylase to visualise adrenergic (autonomic) cells, as well as Hoechst nuclear stain H33258 for total cell counts. In a typical experiment, addition of BDNF caused a 22-fold increase in clonal number of BDNF (3+1) sensory (p<0.001), addition of NGF caused a 4-9 fold increase (p<0.01), whereas the presence of both BDNF and NGF, a 76 fold increase was observed (p<0.001). The total number of cells per colony did not significantly vary under the studied conditions (p=0.8) to 0.89). A newly observed, rare (0.02-0.03%) cell type, BDNF+/NFP-, expressed both autonomic and sensory traits. These cells developed in the presence of NGF and BDNF and were most numerous when both factors were present. BDNF+NFP-1 (autonomic) cells developed exclusively in the presence of NGF. Preliminary data indicate that the embryo contains a limitin-independent cell type, in the presence of which virtually all unpregnated crest cells in dorsal culture express NFP, even in the absence of added BDNF and NGF. The results suggest a) that BDNF directs pluripotent neural crest cells or their immediate progeny to differentiate along the sensory neuron lineage, b) that NGF has a similar but less pronounced effect, c) that the stimulatory effect of both factors most likely is additive, d) that NGF may be for director development of crest cells to develop along the autonomic cell lineage, and e) that a neural tube- or neural crest-derived cell type produces a strong signal that supports the formation of cells developing along the sensory neuron lineage. Supported by U54 NS grant HD04123 and a research grant from the Dysautonomia Foundation.

52.4.11 CATECHOLAMINERGIC (CA) DIFFERENTIATION IN EMBRYOTROPHIC RAT CRANIAL SENSORY GANGLIA: POSSIBLE ROLE OF NERVE GROWTH FACTOR (NGF). D. M. Katz and L. M. Eckert. Dept. of Pharmacology and Neuroendocrinology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

Tyrosine hydroxylase (TH) is transiently expressed by a large subpopulation of C-crest-derived and petroliganglion (PG) cells) cells between embryonic days (E) 11.5 and 15.5 in vivo (Jonakait, et al., 1984; Katz and Erb, 1990). In TH, cells are only rarely observed in other cranial sensory ganglia such as the jugular sinus (JSG) of the glosopharyngeal and vagal nerves. Consequently, we asked whether or not this expression was due to the presence of cells with CA potential in the JSG. Although mechanisms regulating transient TH expression in embryonic sensory ganglia are unknown, we recently found that 15-20% of embryonic CA neurons in the JSG of 13.5 day E15.5 PG neurons in culture increase the proportion of TH cells. In the present study, we therefore, examined whether exposure of JSG to NGF to NGN in culture stimulates the expression of TH in JSG, cells. Explant and dissociated cultures of E13.5-14.5 JSG were grow in 24 hours in the presence or absence of 25 ng/ml NGF and monitored to TH and neurofilament (NF) protein expression by immunochemical staining. In contrast to the JSG in vivo and in control cultures, at least 20% of JSG cell gap in NGF-treated cultures were TH-, indicating the presence of NGF-responsive neurons or neuroblasts with CA potential. These findings raise the possibility, therefore, that the ganglion-specific pattern of transient TH expression observed in vivo may reflect regional differences in tonic regulatory influences, such as NGF. Supported by HL-42131 (DMK).
525.3

THE CALCIUM CURRENT IN THE PRESYNAPTIC NERVE TERMINAL OF THE CHICK GIANT SYANPSE IS INSENSITIVE TO THE DIHYDROPYRIDINE NIFEDIPINE E.F. Stanley and Aisar H. Atrakchi. LB NINCDS, Bld. 9 Rm 1E124, NIH, Bethesda MD 20892.

We have used the cholinergic chick ciliary ganglion calyx-synapse to test the effect of the DHP nifedipine on the calcium current (I_{Ca}) recorded directly from a presynaptic nerve terminal (c.f. Brain Res. 505:341). We first used the whole-cell voltage clamp technique in a control neuron to define the holding potential (-50 mV) at which L-type calcium channels are blocked by 10 μM nifedipine. We then used the same conditions to test the effect of the DHP on I_{Ca} recorded from the presynaptic calyx terminal. Locally applied nifedipine did not reduce the calcium I_{Ca} neither did it block chemical transmission through the ganglion. We conclude that the predominant calcium channel in this presynaptic nerve terminal is not DHP-block sensitive and, hence, can not be characterized as L-type.

525.5


Tetramethylrhodamine-conjugated u-conotoxin (TRuCT; Science 244: 1189, 1989) was used to examine the distribution of voltage-gated calcium channels on motor nerve terminals in the sartorius muscles of Xenopus laevis. In most experiments acetylcholine receptors were also stained with fluorescein-conjugated anti-choline-acetyltransferase. TRuCT blocked neuromuscular transmission and stained neuromuscular junctions faintly. In face views the fluorescence consisted of discrete thin transverse bands at intervals of about 1 μm along the length of the synapse. In side views it consisted of discrete dots, also at 1 μm intervals. This staining was not observed when muscles were pretreated with uCT or when the motor nerve terminals were made to degenerate by nerve resection. Additional observations indicated that the TRuCT staining was restricted to the synaptic side of the nerve terminals and aligned with the functional folds. It is concluded that voltage-gated calcium channels on frog motor nerve terminals are clustered at active zones. This distribution ensures that during the presynaptic action potential intraterminal Ca_{2+} reaches its peak concentration at the same sites where synaptic vesicles are clustered. (Supported by NRC of Canada and by NIH).

525.6

DIFFERENTIAL BEHAVIOR OF SOMA AND GROWTH CONE OF SYMPATHETIC NEURON. Arun R. Valadker, S. Shave, A. Blayev, T. Valadker & D. Fraynare. Dept. of Pharmacology, Wayne State Univ. School of Medicine, Detroit, MI 48201.

Neuronal cell bodies have become popular structures to study Ca_{2+} homostasis by applying the techniques of electrophysiology and fluorescence microscopy. Observations derived from these studies are used to explain the role of Ca_{2+} in transmitter release. We demonstrate that Ca_{2+} is handled differently in the cell body and growth cone, and that changes in Ca_{2+} in the cell body are not reflected in release of β-hormepinephrine (H-BE) from cultured sympathetic neurons. Neurons were examined in electrical stimulation (30 pulses at 2Hz) produced an almost equal rise in Ca_{2+} in the cell body and growth cone, but it’s removal was much faster and complete in the growth cone. Substitution of Ca_{2+} by Ba_{2+} enhanced current in the cell body but not in the growth cone. Ca_{2+} (10 μM) enhanced cell body Ca_{2+} but did not change H-BE release. Although cadmium and verapamil (50 μM) completely blocked Ca_{2+} transport in the cell body these agents produced only partial inhibition (60-70%) of H-BE release even when used in high concentrations (200 μM). We conclude that examination of Ca_{2+} movements only in the cell body is insufficient to understand the role of release of transmitter and its modulation by pharmacological agents.

525.7

TWO PHARMACOLOGICALLY DISTINCT Ca_{2+} STORES WHICH MODIFY (Ca_{2+}) ELEVATIONS PRODUCED BY DEPOLARIZATION IN SYMPATHETIC NEURONS. D.D. Fred and B.W. Tseng, Department of Molecular and Cellular Physiology, Stanford Univ. School of Medicine, Stanford, CA 94305.

We studied effects of intracellular compartments on [Ca_{2+}] responses elicited by 50 mM K+ in bullfrog sympathetic neurons, focusing on the compartmentations from two sources: a ryanodine (Ry) and a calcium (Ca)-sensitive store (SS) and a RY and a Ca-sensitive store (RS). Either store appears to act as a Ca source or sink depending on its Ca content and [Ca_{2+}]. Ryanodine was used to study the ability of the SS to act as a Ca source. Responses elicited in the presence of 1 μM ryanodine (Ry), which inhibits Ca_{2+} release, were slower in onset but otherwise similar to controls (C), suggesting that the K+-induced [Ca_{2+}] rise was Ca_{2+} insensitive to Ry. In contrast to the Ry [Ca_{2+}] responses elicited in the continued presence of Ry. This results for the slow onset (and fast recovery) in (a) that under these conditions Ry did not affect Ca_{2+} levels in the cell. The other store (RS) appears to prolong recovery by releasing Ca_{2+} that accumulates when [Ca_{2+}] is elevated, producing a ryanodine-insensitive plateau (compare a-b) and reducing the plateau during the plateau (d), [Ca_{2+}] rises transiently; after Ry is removed, [Ca_{2+}] falls and undergoes a second transient rise, even in the absence of external Ca. suggesting that the plateau arises from release of Ca_{2+} from an internal source. The increase of 1 μM FCCP, K-elux (C) responses with no obvious plateau, suggesting that Ca_{2+} uptake and release by this store is sensitive to FCCP. Acting together, the SS and the Ry may shape stimulus-evoked [Ca_{2+}] transient(s) in a way that reflect the cells’ history of stimulation.

525.8


The L-type Ca channel was studied in the patch clamp configuration. Y-shaped patch clamp isoprenized cells with a pipette containing pipette solutions to study Ca_{2+} currents in the presence of Ca_{2+} channel blockers. Single Ca_{2+} currents were evoked by applying Ca_{2+} and Ca_{2+} removal to the patch. Ca_{2+} removal decreases the patch size of the Ca_{2+} channel without affecting g_{Ca}, and the inactivation is Ca_{2+} dependent. These experiments provide a direct observation of Ca_{2+}-dependent inactivation of Ca_{2+} channels in intact cells.
CALCIUM CHANNEL SUBTYPES IN ACUTE ISOLATED ADULT RAT AND FROG SENSORY NEURON SOMATA. B.S. Spector et al., Department of Pharmacology and Physiology, University of Chicago, Chicago, IL 60637

Dhdytrophines (DHP) and omega-conotoxin GVa (ω-CgTX) were tested on Ca²⁺ currents recorded from acute isolated rat and frog dorsal root ganglion cells. By ω-CgTX increased peak current in rat cells by 56% ± 12 SE (N=5). Sequential treatment with 2µM nimodipine and then 1µM ω-CgTX totally blocked current in rat cells. The CBF, which is more effective, nimodipine less effective, and more current was left unblocked in cells held at -60mV versus -60mV (Table below).

<table>
<thead>
<tr>
<th>Species</th>
<th>Holding Potential</th>
<th>% Decrease 2µM Nimodipine</th>
<th>% Decrease 1µM ω-CgTX</th>
<th>% Current Remaining</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frog</td>
<td>-60mV</td>
<td>50 ± 4.8 SE</td>
<td>47 ± 4.0 SE</td>
<td>2 ± 1.8 SE</td>
<td>7</td>
</tr>
<tr>
<td>Frog</td>
<td>-80mV</td>
<td>14 ± 3.4 SE</td>
<td>71 ± 3.7 SE</td>
<td>12 ± 2.0 SE</td>
<td>6</td>
</tr>
<tr>
<td>Rat</td>
<td>-60mV</td>
<td>64 ± 4.1 SE</td>
<td>20 ± 2.7 SE</td>
<td>8 ± 2.0 SE</td>
<td>5</td>
</tr>
<tr>
<td>Rat</td>
<td>-80mV</td>
<td>26 ± 4.0 SE</td>
<td>43 ± 3.6 SE</td>
<td>22 ± 2.6 SE</td>
<td>10</td>
</tr>
</tbody>
</table>

The increase in ω-CgTX induced block at negative holding potentials may reflect an increase in the number of primed N channels. Since changing the holding potential from -60mV to -90mV increased peak current by 50% (N=5), the proportion of current blocked by nimodipine at -80mV is less than the expected (50% decrease in rat, 28% decrease in frog) based on the effect of nimodipine observed at -60mV. Thus, the unblocked current may reflect a decrease in efficacy of DHF antagonists on L channels at negative holding potentials. More unblocked current was observed in rat than in frog which may reflect the presence of DHF and ω-CgTX resistant channels in the rat cells.

mRNA REGULATION: PEPTIDES AND C-FOS

ESTROGEN ($) INFLUENCES ON OXYTOCIN mRNA EXPRESSION IN PREOPTIC AND ANTERIOR HIPOTHALAMIC REGIONS STUDIED BY IN SITU HYBRIDIZATION. S.K. Chung and D.W. Pfaff, The Rockefeller University, New York, NY 10021

In order to analyze possible influences of E on oxytocin mRNA expression at preoptic and anterior hypothalamic levels of the rat brain, in situ hybridization was used, supported by immunocytochemistry and compared to vasopressin mRNA in situ hybridisation. A tritiated 25 base oligomer was used, previously confirmed as oxytocin-specific (Kawata et al., Brain Res. Bull., 1988). Ovariectomized female rats were treated for 2 days or 2 months with estradiol (100µg, mixed in cholesterol) or cholesterol alone as subcutaneous injection (sum length). Oxytocin mRNA in situ hybridisation and immunochemistry both showed neurons expressing oxytocin mRNA (Mol, ACB, NAc) that increased in subcutaneous estrogen, but not in subcutaneous cholesterol (about 2X) after 2 days of E treatment, this was not significant. When the amount of oxytocin mRNA per labelled neuron was quantified treatment (either 2 days or 2 months) was shown to significantly increase oxytocin expression in SON and ACB, approximately doubling pixels per neuron. The E effect only occurred in area of oxytocin gene, but it could also be secondary to changes in release of the peptide.

TRANSFORMATION FACTORS BINDING TO THE PREPROENKEPHALIN PROMOTER: EXPRESSION IN NEURONS OF NORMAL AND STIMULATED NUCLEUS CAUDALIS. G.R. Uhl, M.G. Buettner, D. Appleye, M.A. Moskowitz and Y. Nishimori, Lab. of Mol. Neurobiology, Hinda/ARC, and Dept. of Neuro. and, Johns Hopkins, Baltimore, MD 21224 and Dept. of Neuro & Neuropath, MGH and HMS, Boston, MA 02114

Neurons expressing preproenkephalin in lamina I and II of the nucleus caudalis display exquisite activity-related changes in preproenkephalin gene expression. A region of the preproenkephalin promoter that may bind and be regulated by a specific set of transcription factors has been identified. To understand possible mechanisms of trans-synaptic control preproenkephalin regulation in these cells, we identified these factors were studied in nucleus caudalis neurons from brains sacrificed after unilateral stimulation of the trigeminal nerve using in situ hybridization with matched oligonucleotide cDNA probes.

Levels of hybridization to Jun B mRNA are greater than those for cJun, with only scattered neurons hybridizing with Jun D probes. Expression of Jun B is also greater than that of cFos, AP2, NF1-A and NF1-red. After primary afferent stimulation, expression of both Jun B and cFos mRNAs is noted in more neurons. These results are consistent with a role for AP-1 factors in the upregulation of preproenkephalin noted after primary afferent stimulation.
526.3 SEX STEROIDS REGULATE THE DEVELOPMENT AND ADULT EXPRESSION OF PRO-OPIN mRNA IN THE ANTERIOR OVOTHELUMINAL NUCLLEUS (AVP-O) OF THE RAT. R.D. Sillman, Oregon Health& Science University, Portland, OR 97206.

Sex steroids are generally believed to exert important regulatory influences on gonadotropin secretion. Recently, Sillman et al. (1995) demonstrated that estradiol and progesterone are required for full expression of pro-OPIN mRNA in the AVP-O nucleus. This expression was identified in the AVP-O nucleus in female rats at estrus, and was absent in males or in females at proestrus or estrus. These results suggest that the regulation of pro-OPIN mRNA by sex steroids may be involved in the feedback regulation of gonadotropin secretion.

526.4 IN SITU DETECTION OF POMC HETERONUCLEAR RNA IN INDIVIDUAL NUCLEI IN RAT BRAIN AND PITUITARY. M.K., Schabes*, I.P. Herman, R.C. Thompson and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

In situ hybridization techniques (ISH) using exon encoding probes to study the regulation of neuroendocrine peptide mRNA expression are well established. However, measurement of cytoplasmic RNA is difficult and limited by the inability to determine absolute or small changes in biosynthetic activity. In contrast, transcription assays measuring the primary gene transcript in the nucleus appear to reflect the transcriptional rate of the gene much more precisely. Recently, Freema et al. (Science, 1986) have succeeded in studying the expression of the POMC gene in individual pituitary cells by ISH using riboprobes specific for intervening sequences (introns). Downstream abundance of the primary transcript and the long exposure times these studies have been tedious.

In this study we located POMC hnRNA both in individual cells of the rat arcuate nucleus and the pituitary gland by ISH. Riboprobes specific for intron A of the POMC primary transcript were labeled either with high specificity 35S or digoxigenin-UTP for non-radioactive detection. Radioactively labeled hybrids could be detected in pituitary nuclei after one week of exposure time and in the arcuate nucleus after one month. The enzymatic detection of the non-radioactive labeled hybrids yielded positively stained cells in the pituitary gland within 24 hours. However, in the arcuate nucleus the latter method failed to produce a detectable signal. While the quantifiability of the non-radioactive approach made it possible to study the optical and morphological resolution and rapid signal detection. At present we are investigating, whether the hnRNA detection by ISH using intron specific probes truly reflect transcriptional activity.

This work was supported by MH42521, NIDA 022414 and U01DK47245.

526.5 LOCALIZATION AND REGULATION OF VASOPRESSIN HETEROTRONUCLEAR RNA IN THE RAT HYPOTHALAMUS. I.P. Herman, T.G. Sherman, M.K.-H. Schaber, and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0726.

We have used a cDNA probe directed against a purely intronic sequence of the rat vasopressin (VP) gene to localize VP heteronuclear (hn) RNA in neurons in the brains of normal and estrogen- or estrogen- and progesterone-treated rats. The hybridization histochemistry. The sequence used for probe construction was the 3' portion of the VP gene corresponding to a single band upon Southern blot analysis of Sprague-Dawley genomic DNA, confirming its specificity for study of pro-VP hnRNA. Standard ISHH procedures using this probe have shown positive signal in the supraoptic nucleus and paraventricular nucleus. In contrast, ISHH using probes directed against exons of pro-VP showed a positive signal in pro-VP mRNA in the supraoptic nucleus and paraventricular nucleus. In the male rat, testosterone has an inhibitory feedback on LH and FSH. The influence of gonadal steroids on gonadotrophs is relatively well understood. In the female pituitary, progesterone has a positive effect on LH and FSH. Recent studies looking at the effect of gonadal steroids on the PDYN peptide, showed that short term exposure (5-30 days) decreased PDYN peptide expression. However, long term castration (30 days) increases PDYN peptide-IR and that testosterone reverses this effect. In the present study, we examined the effects of short and long term castration on anterior lobe PDYN mRNA. Male rats (200-225 g) were castrated for periods of 5 days, 14 days, 30 days. In one experiment, testosterone (2x day, 100 μg/100 g body weight) was added 7 days after the castration surgery. The signal intensity of PDYN in the nucleus was equal to controls, and total RNA was extracted and submitted to Northern gel analysis. A significant increase in PDYN mRNA was seen in rats castrated for 5, 14 or 30 days. Testosterone reversed the effects of castration, returning PDYN mRNA to normal levels. Interestingly, sham-operated rats significantly lower levels of PDYN mRNA than sham-operated rats. Our data indicate that anterior pituitary PDYN is under the inhibitory influence of gonadal steroids and that removal of this feedback mechanism activates its synthesis activity. Supported by the Theophile Raphael Fund and NIH grant MH 42221. R.D. is a fellow of the Medical Research Council (MRC) of Canada.

526.6 EFFECT OF GONADAL STEROIDS ON PITUARY PRODYNORPHIN mRNA LEVELS IN THE MALE RAT. R. Day, M. Hoyersten* and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI, 48109-0726.

Prodynorphin (PDYN) is the precursor of a series of well characterized opioid peptides with neuroendocrine functions which include dynorphin A 1-17, dynorphin B and α-endorphin. In the rat pituitary, PDYN derived peptide-immunoreactivity (IR) has been detected in the anterior pituitary, co-localized with PDYN mRNA in gonadotrophs, the cells known to produce LH and FSH. The influence of gonadal steroids on PDYN expression in the rat has been conflicting. A recent study has reported that gonadal steroids increase PDYN IR in the hypothalamus of the male rat, whereas testosterone decreases PDYN mRNA expression in the pituitary of the female rat. The effect of gonadal steroids on PDYN expression in the anterior lobe of the rat testis has not been studied. In this study we measured the expression of PDYN mRNA by in situ hybridization in the anterior pituitary in rats with intact testes, castrate, and castrated for 1, 5 and 30 days. In intact rats, PDYN mRNA was detected in gonadotrophs. The signal intensity of PDYN mRNA was lower in castrated rats. After 30 days, PDYN mRNA expression was further decreased. We conclude that gonadal steroids are involved in the control of PDYN expression in the anterior pituitary.

526.7 DIFFERENTIAL EXPRESSION AND DEVELOPMENTAL REGULATION OF TACHYKININ RECEPTOR mRNAs IN THE RAT CENTRAL NERVOUS SYSTEM. J.E. Krause, A.D. Herhey, Y. Takei, and J.P. Sciamanna. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110 and Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46223.

We are interested in the functions and mechanisms of action of tachykinin peptides in the rat central nervous system (CNS). Toward this end, we cloned CDNA encoding a rat substance P receptor (SPR, NK-1 type) and a rat neurokinin A receptor (NKR, NK-2 type). In these studies, we have addressed some issues of expression of these mRNAs in discrete CNS regions and during CNS development. The mRNAs encoding these tachykinin receptors are low abundance specifically. However, sensitive nuclear localization protection assays were established for receptor mRNA detection and quantitation. SPR is a widely expressed throughout the adult CNS, with high level present in striatum, hippocampus and midbrain. SPR mRNA was detected in many other CNS regions, and overall the levels of abundance in CNS regions ranged from 0.01-0.1% that of the present (striatum) to 0.0008% (cerebellum) of total RNA. NKR mRNA was detectable only in striatum and hippocampus, and these mRNA levels were at least 30 times greater for SPR mRNA. The expression of SPR mRNA is developmentally regulated in whole brain and striatal RNA, with detectable levels present at E14 and levels greater than adult were present from embryonic day 18 to postnatal day 21. Similar studies are currently undertaken for NKAR mRNA.

Overall, these SPR and NKR mRNAs are of extremely low abundance in the CNS, 2) the mRNAs show region specific patterns of expression and 3) they are developmentally regulated.
256.9

The cornea has the most densely innervated epithelial surface in the body. Previous studies from this laboratory have shown that removal of a 1mm, 150-200 um thick disc of tissue from the rabbit cornea elicits free nerve endings and the underlying subepithelial plexus while inducing the formation of a dense ring of collateral sprouts by 24 hrs (Rozsa, Ouss, Beuerman, Invest Ophthalmic Vis Sci 24:1980,1033-1051). Sensory signals from the endings in the epithelial wound show a loss of modality specificity, spontaneous activity, and an increase in action potential output to a given stimulus. The present study has investigated gene expression in the same area after wounding by analysis of the mRNA of the rabbit trigeminal ganglion at 0, 15, 30, 45 min, and 1 hr wounding. Total cellular RNA was extracted denatured and dotted on nitrocellulose. The polymerase chain reaction (PCR) was used to detect c-fos mRNA and dot blots of RNA and PCR products were hybridized with 52p labeled c-fos plasmid (ATCC #41042). Compared with RNA extracted from cortex of the same animals, and trigeminal ganglia from un wounded animals the level of c-fos mRNA rose to a peak at 15 min following wounding, and was in decline by 1 hr. The change in electrical activity following corneal wounding may lead to the increased expression of c-fos message in this sensory ganglia. (EYO4074, EVO2031)

256.11
AMPLIFICATION OF REGULATED mRNA FROM OPIATE-TREATED CELLS. S.A. Meeker & J.J. Eberwine, Dept of Pharmacology, Univ. of Pa. School of Medicine, Phila, PA 19104.

We have used in situ hybridization (ISH) and a cRNA probe to study the effect of opiate stimulation on mRNA content in neurons. We use this technique to observe changes in mRNA content of a specific class of neurons after exposure to drugs and to relate these changes to the behavioral effects of the drug.

256.12

Removal of cranial bones is a prerequisite for stereotaxic operations. We have observed that this procedure increases the concentration of mRNA coding for proenkephalin (CCK-mRNA) and preproenkephalin in the rat cortex (Olek and Meyer, Neuropharmacology, 15:115, 1976). We have now extended these studies to show that a second rapid increase occurs in the area of the sensory nerve receptors after the removal of bone, and further that the CCK-mRNA content of the area is lower in the unlesioned cortex.

256.13
ELEVATION OF STRIATAL C-FOS mRNA AND API COMPLEX FORMATION AFTER TREATMENT WITH COCAINE. M.J. ladarco, C.L. Youn*, Y. Hoo, and J.P. Quinl. Neurobiology and Anesthesiology Branch NIEH and *Laboratory of Pathology, NCI, NIH, Bethesda MD.

Levels of Fos protein and Fos-related antigens are elevated by indirect acting dopamineergic agonists (e.g. cocaine) acting through D1 receptors. The present experiments address the relationship of protein expression to c-fos mRNAs and ability of tissue extracts to reconstitute a c-fos/cpn (API) complex. mRNA blot analysis was performed to measure the levels of c-fos mRNA in caudate, frontal cortex and hippocampus. API complex formation was assessed by gel mobility shift assays using extracts from these brain regions and an oligonucleotide from the gibbon ape leukemia virus enhancer (GALV) which contains the API consensus sequence (TAGTGC). Cocaine (5 to mg/kg, ip) produced dose-related increases in c-fos mRNA of up to 8 fold within 30 min. The most marked increases were observed in striatum, consistent with its dense dopamineergic innervation. The cerebellum also consistently showed an increase in c-fos mRNA while little or no alteration occurred in frontal cortex or hippocampus. Gel shift analysis co-isolated all neuronal proteins capable of forming an API complex with GALV. An additional mobility shift of the API complex was consistently observed upon addition of an antibody to c-fos. While the vein expression of c-fos gene expression is thus enhanced, there was no evidence that this increase suggests a role for specific Fos proteins in the regulation of neuronal genes containing API sites.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

257.1

Recent in vivo studies from our laboratory suggest that magnesium ions (Mg2+) can alter neurotransmitter binding and vascular reactivity in the peripheral microcirculation. The present studies were undertaken to determine if Mg2+ influence AR and V tone and reactivity in the rat cortical (pial) microcirculation in vivo. Quantitative, high-resolution television-image intensification was utilized to perform the microcirculatory studies. Perfusion of the rat cerebral hemisphere with BSF containing reduced magnesium concentrations (1-1.6 mmol/L) decreased aortic and femoral blood pressure in an age-dependent manner, with little or no change in cycle length. Perfusion of isolated rat cerebral microvessels with BSF containing reduced concentrations of Mg2+ (1.6 mmol/L) produced a dose-dependent vasoconstriction of the cerebral arteries. The results suggest that Mg2+ exerts a direct effect on the cerebral microcirculation and may play an important role in the genesis of ischemic brain injury.

257.2

Turtles are extremely anoxic tolerant being able to survive for over six months while overwintering in anoxic pond bottoms. This strategy has lead to the brain being used as a model for anoxia studies. Intracellular recordings from slices of rat and turtle cortex were obtained during various anoxic and ischemic conditions. The spinal cord of the turtle seemed to be restricted to the ipsilateral cortical areas. The activity of glutamate decarboxylase, the rate limiting enzyme of GABA synthesis, was not affected in rat cortex after parietal bone removal indicating that the operation did not activate all GABA-interneurons, but only the subpopulation which contains the peptides. Parietal bone removal greatly enhanced ipsilaterally the concentration of c-fos mRNA in a time dependent manner. Already after 60 min, mRNA levels of the prote-oxygen were elevated as compared to the control side. They declined thereafter to be enhanced again after 24 hours. The distribution of cells containing c-fos mRNA in rat cortex was similar to that of COX-containing neurons.

These findings indicate that the prote-oxygen c-fos may play a mediator role in the changes in gene expression of cortical peptide neurons.

**ISCHEMIA VII**
573.3
MCAO in the rat produces an initial hemispheric swelling and infarction followed by a substantial decrease in hemispheric volume. Histologic analysis was performed to elucidate the mechanisms of these changes. Microsurgical techniques were used to produce MCAO in spontaneously hypertensive rats. Paraffin sections of forebrains were prepared at 1, 2, and 15 days post-occlusion and stained with hematoxylin and eosin, or immunohistochemically stained for GFAP. Initially (days 1 and 2), a well demarcated infarct was observed in the cortex with necrosis extending from the meningeal surface to the radius of the corpus callosum. A zone of glial activation (with increased GFAP positivity) was present at the medullary boundary with surviving brain tissue. Polymorphonuclear cells (PMN) infiltrating the meningeal and meningeal vascularity were present around the periphery of the infarct. By day 5, the infarct was beginning to cavitate along its medullary aspect. Macrophages and PMNs were more abundant, especially in discrete foci just below the meninges. By day 15, the once necrotic tissue was replaced by a fluid filled cavity. The mesencephalon over the leision was thickened and attached to a layer of loose connective tissue containing fibroblasts and macrophages. The medial aspect of the cyst was walled off by GFAP positive glia. Ischemic changes were absent in contralateral hemispheres and in sham-operated control animals. These results indicate that the initial hemispheric edema and infarction produced by MCAO are associated with necrosis, PMN infiltration and diffuse gliosis. The later loss of hemispheric volume results from phagocytosis of the necrotic tissue, concurrent with the formation of the glial scar.

573.5
CHARACTERIZATION OF PHOSPHOLIPASE A2 (PLA2) ACTIVITY IN GERBIL BRAIN AFTER ISCHEMIA AND REPERFUSION. G.A. Ridgway*, R.A. Nenestov*, V. Uemura* and J.V. Bently. Departments of Medicine and Neurosurgery, Massachusetts General Hospital, Harvard Med. Sch., Boston, MA 02114
PLA2 activity has been proposed as an important mechanism of ischemic brain injury based upon studies in which PLA2 activity has been measured indirectly from tissue arachidonic acid (AA) release. We characterized the enzymatic activity of gerbil brain after 10 min. of common carotid artery occlusion, followed by 10 min of reperfusion. Cytosolic, mitochondrial and microsomal fractions were prepared by homogenization of forebrain homogenates. PLA2 activity was assayed by release of AA from exogenous 14CJA-phosphatidylcholine. Fractions from ischemic-reperfusion brains had significantly higher specific PLA2 activities (pmol/mg protein/min): Cytosol: Mitochondrial: Microsomal Control: 4.3±0.4: 7.7±0.6: 2.2±0.2 Ischemic: 6.7±0.7 (p<.001) 13.9±1.0 (p<.001) 3.0±0.2 (p<.05)
PLA2 activity was then fractionated by gel filtration chromatography. Two forms of this enzyme were identified: cytosolic and membrane (mitochondrial, microsomal)-associated forms, migrating at approximately 60KDa and 12-14 KDa, respectively. PLA2 activities of both forms were Ca2+ dependent with optimal activities at pH 8.5.
In conclusion, ischemia results in a stable activation of two distinct forms of brain PLA2, a soluble and membrane-associated form. This stable activation of PLA2 may play a major role in cellular injury associated with ischemia and reperfusion.

573.7
Previous studies have demonstrated that dextromethorphan (DM) is neuroprotective in animal models of cerebral ischemia and it has been suggested that this effect is related to noncompetitive antagonism of neuronal NMDA receptors. We studied the effect of cerebral blood flow (CBF) and cerebral injury in a rabbit model of transient, focal ischemia. Rabbits underwent two hour occlusion of the left internal carotid middle cerebral and anterior cerebral arteries, followed by four hours of reperfusion. Ten minutes after the onset of ischemia they were treated either i.v. DM (n=6) 20 mg/kg followed by 10 mg/kg/hr, or normal saline (NS, n=5). Regional cerebral blood flow (rCBF) was measured continuously using a laser Doppler flow meter (TSI) and in some animals with radioactive microspheres. DM attenuated the sharp, post-ischemic rise in rCBF seen with reperfusion in the ischemic core of NS controls (DM 42% pre-ischemic values; NS 108%; p<.05). Preliminary data indicate that DM may also prevent the delayed, post-ischemic hyperfusion that occurs in the ischemic penumbra. DM treated animals demonstrated recovery of the somatosensory evoked potential, compared with NS controls (DM 77% pre-ischemic values; NS 20%; p<.05). DM's effects on CBF may contribute to its neuroprotective action or alternatively, the CBF changes may be secondary to prevention of neuronal excitotoxicity.

574.4
Platelet activating factor (PAF) is a potent mediator of inflammatory responses in various tissues. In the current report, PAF content in brain micro-dialysate samples was assenced acutely after focal brain injury (MRI+TAG laser damage, K.U. et al, Exp. Neurol., 1990) by a specific PAF-radioimmunoassay (DuPont). Dialysis probes (Carnegie) were inserted into the parietal cerebral area of progressive neuronal damage was present at the lesion core as well as into a remote control area in the parietal cortex of anesthetized rats (n=7). No detectable PAF levels were found under baseline conditions prior to injury. Acutely after injury, PAF levels in the dialysate from the injury site were 0.3±0.34ng/ml (p<.05) and remained elevated throughout the observation period of 60 min (0.32±0.09ng/ml p<.05), while no PAF activity could be detected in the remote sampling site.
These data support the hypothesis that PAF, produced by the parenchymal tissue, may trigger early events in the sequence of progressive neuronal death following cerebral ischemia and neurotrauma.

577.6
BLOOD FLOW IN THE BORDEROZONE OF FOCAL CEREBRAL INFARCTS IN RATS. J. Jacewicz, J. Tanabe*, K. W. Wang and V. Polisillani. Dept. of Neurology and Neuroscience, Cornell University Medical Center, New York, NY 10021
Spontaneously hypertensive rats (SHR) and Fisher 344 (F344) rats had their right middle cerebral and common carotid arteries occluded under halothane anesthesia to induce focal, graded cortical ischemia (Brint et al., 1988). The rats awake, and 24 hr later, they underwent a quantitative CBF study (*3H-iodoiodine auteradiography and simultaneous histologic analysis (H&E staining). CBF at the infarct border moved from 20% under baseline to 50 ml/100g/min in controls, and 60% in the sham operated group. CBF (mean±SD in ml/100g/min) SHR (N=7) F344 (N=8) 0.5 mm within the infarct 31±12* 32±15* At the infarct border 44±19 48±20 0.5 mm outside the infarct 74±25* 61±25* Left (nonischemic) cortex 128±29 --- *p<0.05, 2 WAY-ANOVA (vs. CBF at the infarct border)
The results are consistent with observations that the volume of cortex with CBF <50 ml/100g/min at 15 min, 1, 2, 3 and 24 hr after ischemia onset approximates the 34 br peripheral infarct volume (Jacewicz et al., 1985, in press). The CBF threshold for ischemic damage exceeds the 10 - 17 ml/100g/min reported for larger mammalian brains (Astrup et al., 1981) and may reflect the higher baseline cerebral metabolism and CBF found in rodents (Kennedy et al., 1978).

577.8
Microsurgical techniques were used to occlude the MCA in spontaneously hypertensive rats. Decreased microcirculatory perfusion after ischemia was verified using Laser-Doppler Flowmetry. MRI experiments were performed 2 days later using a GE 1.5T whole body imaging unit. Images of 3mm coronal sections were collected using a spin-echo pulse sequence having a TR of 2.5s and a TE of 80ms. Forebrains then were sectioned coronally (2mm), stained with 1% triphenyltetrazolium (TTC), photographed, and fixed by immersion in 10% buffered formalin. Pulsesions were embedded, cut (6 μm) and stained with hematoxylin and eosin (H&E). MRI images and corresponding stained sections were analyzed for the quantification of ipsilateral hemispheric swelling and infarct size using image analysis (Ameresham RAS 3000). Morphological changes quantified using MRI paralleled those observed in stained sections. Ipsilateral hemispheric swelling of matched sections for MRI, TTC and H&E (range 7.1±3% to 9.8±3%) were similar and significantly correlated (r=0.75 to 0.70; p<0.05). Hemispheric infarct size of matched sections for MRI, TTC and H&E (range 38.1±3.1% to 32.9±3.7%, p<0.05) were highly correlated (r=0.94 to 0.90, p<0.001). The identification of infarction by MRI was obvious with the MRI signal intensity in the infarcted cortex being much greater than in the normal contralateral area (increased Signal Intensity p<0.05) and much less in sham operated animals. These data indicate that quantitative MRI can non-invasively detect ischemic tissue damage with results that are well correlated to those obtained by histologic methods.
557.9 TEMPORAL ASSESSMENT OF NMR T2 RELAXATION TIMES AND DIFFUSION COEFFICIENTS OF WATER IN ISCHEMIC RAT BRAIN. R.J. Ordidge 1, 2, R. Wright 1, A. Hoppen 1, M. Chopp 1, L.C. Rodinoff 1, 2 Department of Neurology, Henry Ford Hospital, Detroit, MI 48302, 1 Department of Physics, Oakland University, Rochester, MI 48309 and 2 Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48103.

The objective of this study was to investigate the utility of NMR imaging for the study of the time course of ischemic damage in brain. Middle cerebral artery occlusion (MCAO) in rats was used as a model of permanent focal ischemia. Approximately 25 animals were studied progressively from 1.5 hours to 1 week following MCAO. T2-weighted images were acquired using variable echo time two-dimensional Fourier transform (2DFT) imaging. Intracerebral hemorrhage (IVH) imaging was used to produce diffusion-weighted images (LeeBihan, D., Breton, E., Lallenand, D., radiology 168:497, 1985).

The data showed a gradual increase in T2 which maximized at 24 hours post MCAO at a level of 150% that of pre-ischemic value. This was followed by a gradual decline towards normal over the following week. T2 values, however, never completely returned to normal. The contralateral side showed no variation in T2.

The diffusion-weighted images demonstrated an immediate decrease in the diffusion constant (D-H, 0 to 50% of normal at the earliest time point studied (1.5 hours). This was followed by a gradual increase toward a normal value at 1 week. The contralateral side showed no variation in D-H, O.

We conclude that changes in D-H, O provide a measure of the early evolution of ischemia. This change in water diffusion is specifically related to changes in the structural integrity of the tissue. In addition, the variation in T2 is similar to temporal evolution to reported increases in tissue water content. Histological studies are presently in progress to establish the origin of these changes in the NMR parameters.

557.10 UNBIASED ESTIMATION OF BRAIN DAMAGE IN A RAT MODEL OF MIDDLE CEREBRAL ARTERY OCCLUSION. S.M. Ernst & A. Meulen. Stereological Research Laboratory, Aarhus University and Neurological Research Laboratory, Rigshospitalet University Hospital, Copenhagen, Denmark.

Rat animal models for studying brain damage in cerebral ischemia have been used for a number of years. However, even though these methods have been highly successful models for showing brain damage due to cerebral ischemia, the quantitative assessment of the results has been poor. Although many attempts have been made to quantify the ischemic size the methodology employed has been biased and highly inefficient. Cavalieri's principle was used to estimate the infarct volume of six rat cerebral hemispheres whose middle cerebral artery had been occluded. The method used approximately ten sections from each hemisphere and an unbiased estimate of the infarct volume could be obtained in five minutes with a coefficient of error of less than five percent.


Inflammatory mediators, such as bradykinin and prostaglandins, which excite and sensitize nociceptive primary afferents, are proposed to bond to cellular receptors and activate intracellular second messengers. We studied the effect of the protein kinase C activating Phorbol12,13-Diptylate (PDB) on slowly conducting afferents in adult cats anesthetized with 3-chloralose. Extracellular single unit recordings were made from 35 slowly conducting units, 17 belonging to group III and 18 to group IV of the medial articular nerve. In regard to their sensitivity to passive movements in the knee joint the afferents were classified in high thresholds and high threshold individuals. PDB in concentrations of 10^-7 up to 10^-3 M was applied by an arterial bolus injection close to the joint. In 49% of the fibers studied (33% of the group III and 44% of the group IV) the discharge behavior was altered by PDB. Three distinct effects could be observed. 1. An enhancement of spontaneous activity dependent on the applied dose of PDB (12 units), 2. an enhancement of responses to passive movements in the joint (6 units), and 3. a decrease of spontaneous activity (3 units). No correlation of the effects with the mechanical threshold could be observed. From these results we conclude that in a population of slowly conducting articular afferents, nociception as well as the peripheral component of hyperalgesia are mediated partly by intracellular mechanisms which involve protein kinase C.


It is accepted that living cells prevent colloidosmotic swelling by the operation of the Na pump. Hence inhibition of the latter should lead to cell swelling and eventual lysis. Testing this hypothesis is important for understanding the mechanisms underlying neuronal volume control and because Na pump inhibition secondary to ATP depletion has been proposed as one of the factors generating cytotoxic brain edema. We have used a potentiometric technique to simultaneously measure transmembrane potential and cell water volume changes using intracellular tetramethylammonium as a volume indicator (Cotton et al, J. Gen. Physiol. 93: 649, 1989). II small (Helix aspersa) neurons were exposed to 1 mM ouabain, a dose expected to inhibit the Na pump. As expected for an electrogenic pump, all cells were depolarized. 5 neurons initially swollen and down-regulated their volume rapidly. The cells started to swell within 1 min of exposure to ouabain, swelling to a maximum of 20 to 35% above their initial volume within less than 30 sec. Then, cell volume decreased (initial rate = -10 to -60 uM/min). Cells not only fully recovered their volume in the presence of ouabain but actually shrank to a maximum of about 10%. 6 neurons showed no detectable cell volume changes in the presence of ouabain.

558.2 MULTIPLE EFFECTS OF HISTAMINE ON ELECTRICAL MEMBRANE PROPERTIES OF TRIGEMINAL ROOT GANGLION NEURONS. B. M. Hutchison, F. Pull, and R. M. Miura. Dept. of Pharmacology and Therapeutics, Univ. of British Columbia, Vancouver, C. Canada, V6T 1W5.

Histamine was applied in vitro slices of trigeminal root ganglion (TRG) as part of an investigation on the ability of endogenous pain producing substances to modulate sensory transmission. Slices (500-1000) were prepared from ganglia of decapitated guinea pigs. Bath applications of histamine (1-200 uM) during intrasomatic recording produced slow, transient depolarizations in 21 neurons (1-30 mV), hyperpolarizations in 6 neurons (1-7 mV), and multiphasic responses in 6 neurons. Eight neurons did not respond despite high doses (1-200 uM). The responding cells (n = 33) varied widely in their sensitivities to histamine although they individually exhibited dose-dependent responses. Input resistance increased during depolarizations (mean = 46%), and decreased during hyperpolarizations (mean = -20%). In those neurons which exhibited afterhyperpolarizations (AHPs) in the spikes evoked by current pulse injections, histamine application reduced AHP amplitude and duration. We suggest that histamine produces the depolarizing response by blocking a resting K-conductance, and the hyperpolarizing response by increasing A- the conductance. The multiphasic responses may indicate the involvement of more than one type of K-channel. The possibility is therefore raised that histamine may modulate sensory signals travelling through the TRG.
528.3


Mechanical allodynia (pain evoked by light tactile stimuli) is often observed in Reflex Sympathetic Dystrophy (RSD). A recent assessment of 8 patients with RSD showed that 1) just detectable sensations evoked by common current electrical stimuli (1 sec trains, 1 muc pulse, 100 Hz) applied to the allodynic area are perceived as pain, but are not nociceptive on the unaffected side; 2) Reaction times to painful electrical stimuli are too fast to be due to C-fiber activity, and 3) Mechanical allodynia and light touch are both absent 19-27 min after initiation of ischemic block at a time when small fiber function (warmth and cold detection) is unaffected. These three lines of evidence indicate that allodynic pain sensations are mediated by AS low threshold mechanoeceptive (AIMT) afferents that usually only mediate innocuous tactile sensations.

However, we have noted a paradox: in several RSD patients mechanical allodynia is relieved by ischemic block at 3-3 min duration, well before empirical detection of any impulsive blockade. Contractions and other motor abnormalities may also accompany the perceptual dysfunction of RSD. In an RSD patient with severe, chronic (24 months) contractions of all 5 toes who received an ischemic block applied to the upper thigh, we observed (and videoed) a complete release of the contractions within 6 min. At this time there was no indication of impaired afferent or efferent transmission - sensory testing failed to show any change in detection thresholds and the patient's toes moved normally.

These results suggest that the perceptual and motor effects of these short-duration ischemic blocks are due to factors other than direct blockade of nerve transmission (e.g., hypoxia, pH change, depression of circulating catecholamines) that may drive or enable peripheral mechanisms necessary for the expression of sensory and motor abnormalities.

528.5

ANTIDROMIC VASODILATATION OVERRIDDEN BY SOMATOSYMPATHETIC REFLEXES IN MAN. INTRANEURAL STIMULATION AND THERMOMETRY. J. Ochoa, D. Yaminsky, P. Marchettini, B. Botto, M. Clino, Good Samaritan Hosp. & Med. Cz. and Oregon Health Sciences Unit, Portland, OR, USA.

Early diffuse cooling of skin in normal hand is consistently elicited by excitation of sensory fibers in peripheral nerve trunks. In turn, delayed regional warming is also consistently recorded, provided stimulation activated nociceptor afferent fibers. We have studied, thermographically, interactions between these two physiological responses, as elicited in healthy volunteers and patients by microstimulation of median and ulnar skin-nerve fascicles. Cooling response starts rapidly after onset of stimulation, affects the entire palmar hand, and is not mediated reflexly. The response disappears after ipsilateral sympathectomy; it is a somatosympathetic vasocostrictor reflex. Warming response surfaces after stimulation is discontinued, once reflex vasocostriction relaxes. It is regionally confined to the receptive field of the stimulated nerve, persists after sympathectomy and disappears with degeneration of small caliber fibers. It is due to antidromic vasodilatation.

Once regional antidromic vasodilatation is established it persists for many minutes, to fade spontaneously thereafter. However, re-stimulation of the nerve rapidly overrides regional vasodilatation through re-engaging reflex vasocostriction. These findings demonstrate predominance of catecholamine-mediated vasocostrictor response over substance P-mediated vasodilator response. It is thus possible that, in nerve disease, antidromic vasodilatation triggered by ectopic discharge in nociceptor fibers may become masked by superimposed reflex sympathetic vasocostrictor activity as a response to orthodromic, afferent, ectopic nerve impulses.

528.7

STIMULATION OF REINAL AFFERENT NERVES INHIBITS CARDIOPULMONARY REFLEX RESPONSES IN THE CONSCIOUS RAT. S.J. Lewis, C. Barres*, H.J. Jacob* and W.J. Brody. Dept. of Pharmacology & Cardiovascular Ctr., Univ. of Iowa, Iowa City, IA 52242.

Recent electrophysiological studies have demonstrated a functional interaction between renal baroreceptor and cardiopulmonary afferents within the nucleus tractus solitarius. The present study examined the effect of renal afferent nerve stimulation (RANS) on the cardiopulmonary reflex (CPR)-mediated cardiovascular (CV) responses, produced by i.v. 5-HT, in conscious shamoperated and renal denervated rats (n=8). The rats were fitted chronically with arterial and venous catheters and an electrode for distal renal aortic denervation (BAD, 14 days post-surgery, n=8). The rats were instrumented chronically with arterial and venous catheters and an electrode for distal renal aortic denervation. The effects of concurrent RANS (0.1 mA, 2 sec, 2.5-2.7 Hz for 10 sec) on the CPR-mediated CV responses were measured. The CV responses were modified by baseline 121 beats/min and again 48 h after contralateral vagotomy. In rats with vagal intact, the CPR-mediated reductions in heart rate and blood pressure were not modified by renal denervation. However, in animals with BAD rats with contralateral vagotomy, RANS virtually abolished the CPR hypothension and bradycardia. These results indicate that afferent renal nerve activity can profoundly inhibit CPR responses by mechanisms that are independent of the baroreflex.

528.8


Norepinephrine (NE) is thought to be a modulator of a modulator of sympathetically maintained pain that may occur following nerve injury. In normal animals however, NE alone has no effect on nociceptive threshold. Recent behavioural studies have demonstrated that NE-induced hyperalgesia can be produced in normal animals if NE is injected in combination with the C*-specific aminophore A23187 on mechanical thresholds of C-fiber mechano- and mechano-neuropathic nociceptors.

Action potentials of single C-fibers were recorded from the superficial pere of porcine abdominal aorta (10mg/kg, ip). Receptive fields were located in the hairy skin of the hindpaw and mechanical thresholds were determined using calibrated von Frey hairs.

Intradermal injections of saline, NE or A23187 alone did not produce sensitization in 12 neurons tested. However, NE+A23187 produced a significant decrease in mechanical threshold (base-line=3.5±1.0 g; p<.05; n=3, 2.5±1.5 g; n=12, p<0.05) with latency to onset of 5-10 min.

These data show that local changes in C*- can induce a state in which NE elicits sensitization of nociceptors. Studies are in progress to address the cellular and molecular events that are involved in this process.

528.9

FUNCTIONAL EVIDENCE THAT 5HT7-RECEPTORS EXIST ON RAT VAGAL AFFERENT PERIKARYA. P.J. Lacolley, S.J. Lewis and W.J. Brody. Dept. of Pharmacology & Cardiovascular Ctr., Univ. of Iowa, Iowa City, IA 52242.

We have shown that administration of serotonin (5-HT) into the occipital artery (OA) blood supply to the nodose ganglia (NG) of urethane-anesthetized rats produces cardiovascular effects via interaction with vagal sensory perikarya (FASER J. 4(3):1117, 1990). The purpose of this study was to determine the 5HT receptor subtypes involved in the actions of 5-HT on sensory perikarya (activated by the OA route) and those on sensory terminals (i.v. route). The initial results with i.v. 5-HT (25 and 3,000 mg/kg) indicated that heart rate response to 5-HT was elicited by the specific 5HT receptor antagonists ketanserin and xylalcin (200 mg/kg, iv) and the mixed 5HT/5HT receptor antagonist metergoline (500 mg/kg, iv). The initial results suggest that 5HT-receptors, present on vagal afferent perikarya within NG, appear to mediate the component of the 5HT-induced reflex evoked by direct activation of these sensory nerve cells.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
SOMATIC AND VISCERAL AFFERENTS IV


In contrast to the rare presence of the catecholamine cell body population in the spinal ganglia, there is a significant number of these cells in the retrograde regions of the DRG in many species. These cells are thought to be responsible for the synthesis and release of dopamine (Supported by grants B, H and I, NIH, Bethesda, MD 50832).

528.10 LABELING OF FOS PROTEIN INCREASES IN AN EXPERIMENTAL MODEL OF PERIPHERAL NEUROPATHY IN THE RAT. K.C. Klauder, S. Waskins*, G. DiRosa* and M.J. Jadad. Department of Otolaryngology, University of Minnesota, Minneapolis, MN 55455, Anesthesiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

An experimental model of a painful peripheral neuropathy has been introduced by Bennett and Xie (1988). The model involves the injection of 4 chronic gut ligatures tied loosely around the common sciatic nerve in rat to produce behavioral signs of neuropathic pain. In models of tissue injury, Fos protein, the product of the c-fos gene, has been used as a marker of activity in spinal cord neurons. We used immunocytochemical techniques in this study to evaluate changes in Fos labeling in the spinal cord at times between 1 and 60 days after injury. The left common sciatic nerve in each of 8 rats was ligated and a sham surgical procedure was performed on the right side. Then at selected times, rats were deeply anesthetized and perfused transcardially with 4% paraformaldehyde. After perfusion, sections from L4-L5 spinal segments were collected (20 μm thick) and stained with the paxillide-antiperoxidase method. Localization of labeled cells were reconstructed using a drawing tube at 10×. There was an increase in nuclear labeling for Fos in the spinal cord on the side of the nerve injury at 3 and 5 days after the injury (p<0.05, Student's t-test). The increase in labeling occurred in both the superficial (I-II) and deep laminae (V-VII). At 14 days, the increase was still apparent but was diminished. There was no side-to-side difference in labeling at 1 or 60 days.


We have proposed a four channel model for tactile based on psychophysical experiments performed on glabrous skin and have subsequently tested this model on the individual channels (Bolanoski et al., JASA, 84, 1988). It is known that hair skin is innervated by a different compliment of receptors than is glabrous skin suggesting that psychophysical experiments on this type of skin may modulate the organization of the different channels of touch. We have used intra dermal injections of 3-cm wide, 0.008 mm thick sections of skin which were used to identify the neurones that change their thresholds that occurred under various conditions: 1) a low-frequency channel (0.4-2 Hz) which is temperature insensitive and is modulated by stimulus size; 2) a mid-frequency channel (2.0-7.0 Hz) which is temperature sensitive and insensitive to changes in skin stimulus size; 3) a high-frequency channel (7.0-50.0 Hz) which is insensitive to temperature changes and insensitive to changes in skin stimulus size. These three channels for hair skin have characteristics that are different than those of the analogous low-, mid-, and high-frequency channels identified in glabrous skin as would be expected from the difference in physiology and anatomy of the two skin types. Whether additional channels exist for hair skin remains to be determined.

REGENERATION: CHAMBERS AND GRAPTS


We have shown that cut adult rat dorsal root ganglion (DRG) axons regenerate into transplants of FSC and form synapses there. The time course over which the regeneration of FSC axons is unknown and it is also unknown whether or not the regenerated FSC axons exist. Since calcealin is related peptide (CGRP) is a marker for dorsal root ganglion axons, it is also a marker for regeneration. In this study we used CGRP immunocytochemistry to label regenerated axons in FSC transplants, and quantitative stereological methods to assess the time course and extent of regeneration. Transplants of embryonic day (E14) spinal cord were introduced into a cavity aspirated in the lumbar enlargement of adult Sprague-Dawley rats. The L4 or L5 dorsal roots were cut and a 1.4 by 4.4 L4 dorsal root was placed into the transplant to form dense bundles by 1 week. The area fraction of the transplant occupied by CGRP-labeled axons increases until 3 months, and then persists unchanged by 3 year. Dorsal roots therefore may reach the FSC transplants before a glial barrier is established, grow for several months within the transplants, and establish an apparently permanent innervation of the transplant recipient without destruction of damaged neural circuits. Supported by VA Medical Research Service, NIH grant NS42707, and USAMRDC grant 5190002.

529.2 ASTROCYTES MAY CONTRIBUTE TO REGENERATION OF DORSAL ROOT (DR) AXONS INTO FETAL CNS TRANSPLANTS. K. Kikuchi, P. Levitt and A. Tealor. Philadelphia VA Medical Center and Departments of Anatomy and Neurology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Transplants of fetal spinal cord support or promote regeneration of severed adult DR axons and allow synapse formation. To analyze the components of the transplants that provide this favorable environment, we studied whether or not astrocytes could mediate dorsal root regeneration. We used calcealin gene-related peptide (CGRP) immunocytochemistry to identify regenerating axons because in normal dorsal horn CGRP specifically labels a population of DR axons. Astrocytes are clearly identified by astrocyte (6-8 μm) and 18 spinal cord or E18 neocortex and labeled with rhodamine microspheres to distinguish them from host astrocytes. Harvested cells (1-6 x 10^6) were transplanted as part of a ventromedial (V) or parasagittal (P) pattern as transplanted in the L4 segment of adult female Sprague-Dawley rats. The L4 or L5 dorsal root was cut and juxtaposed to the transplant. One month later sagittal cryostat sections were processed for both Zorn immunocytochemistry and labeled axons regenerating into all types of fetal astrocyte grafts, but were also identified in central transplants consisting of either Marigel or plasma clot. These results indicate that astrocytes regenerate in response to various environments and suggest that one common feature is the presence of inhibitory influences known to be present in the adult mammalian CNS. Supported by VA Medical Research Service, NIH-NR-24707, and USAMRDC grant 5190002.
529.3 SMALL DIAMETER CARBON FILAMENTS IMPLANTED INTO THE TRANSECTED SPINAL CORD OF THE ADULT RAT SUPPORT AXONAL GROWTH AS DEMONSTRATED BY IMMUNOFLORESCENT TECHNOLOGY. J. REGENERATION, T. Khan, S. Sayer*, R. Haueter*, G. Guik, and K. Barker*. Neuro-Regeneration Lab., RR&D Center, VA Hospital, Hines, IL 60141. Axons that regenerated between the transection gap were immunostained with a monoclonal antibody against neurofilament (ARCs). Carbon filaments have been shown to support the growth of embryonic spinal cord in tissue culture (Khan, T. et al. Abstr. Soc. Neurosci. 11:586, 1985) and to function as a "scaffolding" for the growth of regenerated axons in spinalized rats (Dauwradt et al. Abstr. Soc. Neurosci. 13:876, 1989). In the present study we undertook to further characterize the cellular processes observed growing into carbon filament implants positioned into the transection gap. Fifteen 20g wistar rats sustained total spinal cord transection at level T8-T9 with the use of a knife blade followed by a 20g fine wire through the transaction site. In ten of these animals the resulting transection gap was then filled with a 5mg long bundle of approximately 10,000 carbon filaments of 0.5μm. Five rats were used as surgical controls. After a six-week survival period cryostat cut sections from both groups of animals were examined for the presence of nerve fibers using an indirect immunofluorescent technique with anti-axon antibody and fluorescent-conjugated IgG as secondary antibody. In surgical control animals, sections taken through the transaction site were devoid of fluorescent label while sections taken through carbon filament implants demonstrated the presence of many neurite-positive axons. Although these findings do not address the origin or course of the neuritization of positive axons, they do demonstrate that small diameter carbon filaments can support and direct the growth of damaged spinal pathways. Supported by funds from Veterans Affairs, Rehabilitation R&D Service, Rehab. R&D Grant 19823R.

529.5 IMMUNOCYTOCHEMICAL CHARACTERIZATION OF CNS AXONAL REGENERATION THROUGH TRANSPANT TRANSPLANTS OF SCHWANN CELL/MATRIX CABLES. R.W. Cohen*, T. Goodnick* and E.F. Komen. Division of Anatomy & Cell Biology, Georgetown University, Washington, DC 20007. Prior experiments demonstrated that forebrain cholinergic axons regenerate through transplants of selectively permeable polymer tubes (ARCs) containing dissociated Schwann cells in an extracellular matrix (ECM) cable. The present study further characterizes this regeneration by identifying specific axonal and glial proteins present on cellular processes within the regenerating regions. For these experiments Schwann cells were dissociated from monolayer cultures obtained from neonatal rat sciatic nerve and triturated with newborn rat tumor derived fibroblasts. They were allowed to form a cable within the ARS before transplantation to the lesioned septo-hippocampal pathway. After survival times of 4-28 days the following observations were made: 1. Immunocytochemically stained sections: Schwann cells consistently stained intensely for the low affinity receptor for nerve growth factor (NGFR) and usually stained for glial fibrillary acidic protein (GFAP), but rarely for S-100. Glial cells from the host CNS that migrated onto the ECM cables stained for GFAP and S-100 but not NGFR. Regenerating axons (stained for GAP-43 and LI) closely associated with the Schwann cells and appeared to fasciculate near them. Much less growth occurred on the ECM cables colonized by CNS glia which lacked NGFR or LI. These results are consistent with the hypothesis that growth factors and cell adhesion molecules help mediate CNS axonal growth in vivo. Supported by NIH grant NS23522 and the American Paralysis Assoc.

529.7 CHARACTERIZATION OF RAT SPINAL CORD CHANGES FOLLOWING PHOTOCHEMICALLY-INDUCED INJURY. A.T. Salvaterra, V.R. Holets and M.B. Bunge. The Miami Project and Departments of Neurological Surgery and Cell Biology and Anatomy, University of Miami School of Medicine, Miami, FL 33136. A model of spinal cord injury in rats was developed using a dye laser in combination with a photodisrupting beam. Focal lesions were generated using shorter wavelengths (500-650νm) and were similar to those observed in laser-induced lesions. Although the resulting lesions were similar to those observed in laser-induced lesions, the resulting lesions were different. These results suggest that the use of different wavelengths may be important in the development of new treatments for spinal cord injury. Supported by funds from the Miami Project and the Daniel Heumann Fund for Spinal Cord Research.

529.1 SOMATOSENSORY EVOKED POTENTIALS RECORDED ACROSS CARBON FILAMENTS IMPLANTED INTO THE SITE OF A COMPLETE SPINAL CORD TRANSECTION IN THE ADULT RAT. E. Holets*, T. Khan, S. Sayer*, M. Dauwradt, and K. Barker. Neuro-Regeneration Lab., RR&D Center, VA Hospital, Hines, IL 60141. Carbon filaments have been shown to support the growth of embryonic spinal cord in tissue culture (Khan, T. et al. Abstr. Soc. Neurosci. 11:586, 1985) and to function as a "scaffolding" for the growth of regenerating axons in spinalized rats (Dauwradt et al. Abstr. Soc. Neurosci. 13:876, 1989). In the present study we undertook to further characterize the cellular processes observed growing into carbon filament implants positioned into the transection gap. Fifteen 20g wistar rats sustained total spinal cord transection at level T8-T9 with the use of a knife blade followed by a 20g fine wire through the transaction site. In ten of these animals the resulting transection gap was then filled with a 5mg long bundle of approximately 10,000 carbon filaments of 0.5μm. Five rats were used as surgical controls. After a six-week survival period cryostat cut sections from both groups of animals were examined for the presence of nerve fibers using an indirect immunofluorescent technique with anti-axon antibody and fluorescent-conjugated IgG as secondary antibody. In surgical control animals, sections taken through the transaction site were devoid of fluorescent label while sections taken through carbon filament implants demonstrated the presence of many neurite-positive axons. Although these findings do not address the origin or course of the neuritization of positive axons, they do demonstrate that small diameter carbon filaments can support and direct the growth of damaged spinal pathways. Supported by funds from Veterans Affairs, Rehabilitation R&D Service, Rehab. R&D Grant 19823R.

529.6 DO AXON-FREE NERVES LEAD TO THE FORMATION OF SCHWANN CELL-CABLES WITHIN SILICONE CHAMBERS? A.A. Zaizov, N.A. Azam and L.B. Williams. Lab. of Neural Control, NIH, NINDS, Bethesda, MD 20892. After suture of proximal and distal stump ends, the gap was filled with the ends of a silicone tube, a tissue cable forms through which axons regenerate. Schwann cells are critical for this phenomenon. If the distal nerve stump is omitted or substituted, a fibrin or matrix graft or transient tissue filling in the tube but axons do not regenerate. In this study, we sought to determine whether axons were needed to induce the formation of nerve cable. The transected stumps of rat sciatic nerves were sutured into silicone chambers prefilled with dialyzed SWAN cells. In a 10mm interstump gap. In order to eliminate axons from entering the chamber, the proximal stump was sutured to the ab-synaptic end of a Silastic non-myelinated nerve. The distal stump was then recorded, leaving a 2mm plug of nerve in the proximal chamber. After four weeks, the growth into the silicone chamber, Light microscopy revealed the presence of blood vessels and cellular elements but the absence of myelin. Electron microscopy demonstrated the presence of Schwann cells and fibroblasts. Surprisingly, there were no surrounding unmyelinated axons. These axon-associated Schwann cells developed a basement membrane whereas those of the distal segment did not. The blood and perineurial-neurive barriers did not form in these cables since the barrier-tracer HRP flowed them in after an IV injection. In other animals bearing a four-week cable, the reflected nerve stump was re-separated into the nerve plug from the proximal end of the chamber. Three months later, the cable and the distal nerve were filled with myelinated axons, and the connective tissue barrier was restored. We uncovered a source of non-myelinated axons from the distal nerve stump that were entrapped in the ab-synaptic end of the silicone cable. A non-myelinated nerve free tissue can form a Schwann cell-cable. Nevertheless, a cable did form that was capable of regenerating proximal nerve stump. A cable formed by our surgical method has the potential to be used clinically for delayed nerve repair.

529.8 AXON GROWTH INTO IMPLANTS OF SCHWANN CELLS PLACED IN LESIONED SPINAL CORD. C.L. Paano and M.B. Bunge. The Miami Project to Cure Paralysis and Dept of Neurosurg. and Cell Biology & Anatomy, University of Miami School of Medicine, Miami, FL 33136. Richardson and coworkers (Nature 264, 80) found that CNS axons regrow into segments of peripheral nerve implanted into the spinal cord but Krehleng and others (J. Comp. Neurol. 267, 145, '87) are characterizing this lesion prefontry to transplantation studies. A central axis develops with regions of the lateral and ventral white matter and ventral horns remaining intact. Light and electron microscopy evaluation of lesioned spinal cords were done at 2, 4, 8, 12, 28 and 56 d, and 6 4 mo post-injion. At 14 d, demyelination and beginning remyelination were evident on the lesions of the cord. The region of injury contained macropores that were less numerous by 28 d. Also at 28 d, numerous thin myelin sheaths of both oligodendrocytes and Schwann cell origin were visible; they occurred side by side. Nonmyelinated axons were still observed. Astrocytic processes formed an intermittent border around the perimeter of the cyst. At 56 the edema and tissue debris inside the cyst had largely been resolved, resulting in a shrunken dorsal cord and an area of ventral white matter that appeared larger than would be expected. Some Schwann cells related to axons were positioned in perivascular spaces. A surprisingly high number of axons myelinated by Schwann cells were seen in more dorsal regions, suggesting the possibility of considerable axonal regrowth with increasing time post-injury. (Funded by the Miami Project and the Daniel Heumann Fund for Spinal Cord Research.)

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
TRANSPANTATION: EXPRESSION OF SPECIFIC NEURONAL MARKERS


Human embryonic retinas (postconceptional age 3-10 wks) were grafted to the retina of immunocompetent adult rats. The donor tissue became engrafted in the host retina. The development of the xenograft was followed from 19 to 57 wks of total age. Immunohistochemistry was performed on Bouin’s fixed parasagittal sections with monoclonal antibodies for Xinrin (dilution 1:1000) and N-CAM (1:50). The graft developed as a whole retina, and the xenograft showed a normal pattern of retinal layers. The ganglion cell bodies were well stained, and the axons of the inner plexiform layer and the axonal sorting of the graft were observed. The graft demonstrated the same characteristics as normal retina.

S50.2 TRANSPLANTED NEURAL RETINA EXPRESSES ANTIGEN SPECIFIC FOR NORMAL DORSAL RETINA. K.T. Vre and R.D. Lund Department of Neurobiology, Anatomy and Cell Science, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261

We have previously shown that embryonic mouse retina transplanted into brains of newborn rats project to appropriate visual target regions and make functional connections. To examine the development of connections by grafts transplanted in vivo, we used a monoclonal antibody specific for dorsal retina (Dolce) which recognizes a 68 kd-laminin receptor during the latter part of embryonic development through embryonic life (Dolce et al., Neurosci. Abs. 15:456, 1989). Staining with this antibody is observed between embryonic day 13-14 but can be seen at diminished levels up to postnatal day 13. In this current study, we have examined if mouse retina transplanted into the brains of rat hosts expresses this antigen.

Donor embryonic CD-1 mouse retina were transplanted into the brains of neonatal Sprague-Dawley rats. Following survival periods of at least 5 days, animals were perfused and fixed for light microscopy. Brains were sectioned and incubated in Dolce with 0.5% Triton. Antibody binding was visualized with an HRP conjugated secondary antibody and a diaminobenzidine nickel intensification reaction.

Our data show staining of cells in a localized region of the transplanted retinas as well as axons emerging from the transplants. This occurs even when the grafts are highly folded. While there can be changes in the overall anatomic configuration of the retina, staining with Dolce is still seen. This data provides further evidence that transplanted retinae develop characteristics of normal retina.

(Supported by NIH HD 07343 and EY 05308)

S50.3 VARIATIONS IN PERIVASCULAR LAMININ IMMUNEACTIVITY DURING BRAIN ANGIOGRAPHY. J. M. Krum and J. M. Rosenblin. Dept. of Anatomy, George Washington University Medical Center, Washington, D.C. 20037.

Changes in the distribution and quantity of laminin within the basement membranes (BM) of rat CNS vasculature during vascularization of fetal neocortical grafts, wound healing and spinal cord development were investigated using two immunocytochemical techniques. In paraffin sections, which were routinely enzyme pre-treated, all vessels reacted with laminin. In contrast, the neocortical transplants in developing brain and the regenerating vessels at transplant and wound sites were strongly immunoreactive without enzymatic pretreatment. In formalin-fixed tissue, laminin immunoreactivity decreased after perivascular astroglial contact occurred. The existence of a variant form of BM structure or differences in neuropil density between developing or injured and intact adult brains might account for these observations. (NIH NS-17448)

S50.4 CALBINDIN D-28K IN EMBRYONIC BASAL FOREBRAIN GRAFTS TO ADULT RATS. S. Shoham and E. Herkenham, Dept. Neurosurgical Research, Sata Herzog Hospital, Jerusalem 91010, Israel.

In Alzheimer’s disease (AD) there is substantial reduction in the number of cells containing the calcium binding protein calbindin D-28K (CB) in cortex and in nucleus basalis of Meynert (nBM) (Ichimiya Y. et al., Brain Res. 472:155, 1989). To examine the relation of CB cells to the cholinergic nBM-cortical system which also degenerates in AD, adult rats received 100,000-200,000 embryonic rat E16 basal forebrain (the primordial nBM), to cortex (methods of Fine et al., Neuroscience 16:769, 1986). After 8-12 months CB neurons were identified as calbindin vasculature. In areas of the host deep prefrontal and cingulate cortex and cholinergic regions by acetylcholinesterase (AChE) histochemistry (7 rats, 14 grafts) in 25um thick sections. There were 37-45 CB cells/mm 2 in AChE-positive regions and 41 CB cells/mm 2 in AChE-negative graft regions. The number of CB cells/mm 2 in areas of the host deep prefrontal and cingulate cortex where grafted grafts were placed. Some CB neurons in AChE patches extended axons into the host cortex. This preliminary evidence suggests a difference in the relation of the cholinergic nBM-cortical system and that CB is expressed in nBM neurons even without their normal effenter inputs from amygdala, striatum and other regions.
P30.5

DEVELOPMENT OF SYNAPSIN I AND SYNAPSIN II IN INTRAOCULAR HIPPOCAMPAL TRANSPLANTS. A.Ch. Granzholm1, E. M. Dudek2, H. Bergstrand1, and M. Browning2. 1Department of Cell Biology, Univ. of Linkoping Fac. Health Sci, Linkoping, Sweden, and 2Department of Pharmacology, Univ. of Colorado Health Sci. Ctr., Denver, Colorado.

Synapsin I and II are synaptic vesicle-associated neuronal phosphoproteins that are thought to play a role in the regulation of neurotransmitter release. The levels of these proteins are low in cortical regions of newborn rats but exhibit a dramatic increase postnatally, parallel to synaptogenesis. The aims of the present study were to evaluate whether hippocampal grafts would follow a normal time course of synaptogenesis. Hippocampal tissue was dissected from rat fetuses of embryonic day 18 and transplanted to the anterior chamber of the eye of adult hosts. Grafts and in situ tissue was homogenised in 1% SDS/solution or fixed and sectioned on a cryostat for immunohistochemistry. The levels of synapsin I and II were assayed by immuno-blot methods. The following time periods were used: fetal day 18, birth, 1, 2, 4 and 8 weeks postnatally. The transplants developed levels of synapsin close to the in situ levels, with no obvious delay due to transplantation. The ratio of synapsin I and II reflected a CNS rather than a PNS ratio. The histological analysis showed a similar development, with accumulations of synapsin-positive profiles adjacent to the pyramidal cell layer. Synapsin levels in aged transplants are under investigation. These studies demonstrate a biochemical evidence of a close to normal development of isolated hippocampal formation transplants in vivo. Supported by the Swedish Medical Research Council grant 8650 and NIH grants AG04418, DK40843 and NS26377.

P30.7


The functional activity of neontal pineal glands grafted into the cerebral hemisphere of littermate rats (0-1 day old) Long Evans, black-hooded) was tested in 4 groups: (1) unoperated controls (C; n=7); (2) pinealectomized rats (PX; n=6); pineal transplants (PT; n=7); and pineal transplants that had been pinealized (PT+PX; n=7). Using hypothermic anesthesia, pineal glands were placed into small cortical lesion cavities made rostral to bregma immediately before grafting. At 4-5 months, rats were chronically cannulated and serum melatonin levels measured by RIA (CIDTech) during the light and dark period of the 24-h L:D cycle (10 h L: 14 h D). A significant decline in melatonin was observed between C vs PX (p<0.05), but not between C vs PT and C vs PT+PX. These findings indicate that pineal transplants were functionally capable of restoring nocturnal levels of serum melatonin. Elevated daytime melatonin levels in the PT+PX group suggests that some transplants may have been free-running.

(Supported by NSF BNS-88-1726, NIH NS-13230)

P30.9


GAP-43 is a neuron-specific phosphoprotein which is differentially regulated during normal development. High levels of this protein are correlated with axonal elongation in-vitro, regeneration in-vivo, and highly plastic neural structures such as the hippocampus and olfactory bulb. Work in our laboratory shows that in the developing rat nigrostriatal pathway GAP-43 immunoreactivity is highest during the 6-day period E15 until birth at which time it declines rapidly to adult levels. This time course corresponds with the period of axonal extension for the majority of the substantia nigra dopaminergic neurons. In the present study we use an antibody against GAP-43 to characterize the time course of development of transplanted fetal mesencephalic neurons. For these experiments 0.5HDA lesions of the nigrostriatal tract were made in Sprague Dawley rats. Adequacy of the lesion was determined by amphetamine-induced rotation prior to the transplantation of pieces of ventral mesencephalon (VM) obtained from E15 fetuses. Immunocytochemistry revealed high levels of GAP-43 at 5 days and high levels at 15 days post transplant but lower levels at 3 weeks. By 13 weeks the immunoreactivity present within the transplant tissue is equivalent to normal background levels within the host neurtube. Thus, the presence of high levels of GAP-43 immunoreactivity is prolonged compared to the developing VM in situ. This suggests that axon elongation occurs over a longer period in VM grafts than in situ. Such retarded graft development could be the result of a number of different phenomena such as: surviving cells whose processes were sheared off during surgery may reinitiate axonal elongation; the altered environment of the host may contain trophic factors in sufficient quantities to support rapid growth; or a lack of normal targets for grafted neurons may extend their growth period in a search for alternatives.

P30.6


GnRH neurons are derived from the olfactory placode and migrate into the CNS during embryogenesis. In this study we examined the capability of transplants containing this migratory population of GnRH cells to establish functional connections with the median eminence (ME), and hence to induce gonadal recovery in mutant hpg mice. Nasal area tissue from normal mouse embryos at E12 or E13 was grafted bilaterally into the anterior hypothalamus or preoptic area of adult hpg males (n=4). Following survival of 2 to 8 weeks, immunohistochemistry was performed on the testes weight recorded, and the brains processed for GnRH immunocytochemistry. In none of the grafts did any GnRH neurons infiltrate the third ventricle. Of these, L28 showed gonadal recovery (testes weight of 23.6 mg, mean weight of untreated hpg testes; 7.5 mg). This recovery corresponded to the presence of a small number of GnRH cells in the graft and a sparse innervation of the ME. L20 had a much more robust ME innervation but due to early sacrifice (20d) gonadal recovery was not yet evident. In those animals where the graft remained wholly within the parenchyma, testicular recovery did not occur. However interesting observations were made. In n31 GnRH cells appeared to migrate out of the graft into the host and these cells elongated and differentiated. These observations suggest that migratory GnRH neurons when grafted into the brain of adult hpg mice retain some migratory potential and can elaborate extensive axonal projections, which upon innervation of the ME can induce gonadal recovery. NS 20335.

P30.8


The effects of delay (8-17 d) between lesion and transplant (TP) and delay (1-4.5 d) between harvest and TP of fetal (E15-E17) cholinergeric cells into the lesion site or the caudate n. (CN) were examined in 16 rats with ibotenic acid lesion of the left n. basalis magnocellularis (NB). Brains were processed for cresyl violet, ChAT- and GFAP-ICC, and for ACHE and cytochrome oxidase (CO) 2 months after TP. Survival was always poor in the CN, with small, immature TP cells, extensive gliosis, and low levels of CO activity. In the NB, the best TP survival was observed for fetal cells implanted less than 2h after harvesting. Healthy TP-neurons displayed robust CO activity. Also an intense cholinergic innervation was observed within these TPs, with TP-cholinergic neurons sending processes throughout the TP. A small astrocytic reaction was noticed within TPs and at the host-TP interface. In about 1/3 of the TPs, Status Marmoratus-like profile was noted. The effects of TPs on the host tissue will also be presented.

Supported by FIDIA and NS01 BS 25685-05

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
TRANSPLANTATION: GENERAL III

531.1
Progress in neural transplantation would be aided by improved monitoring of the fate of donor tissue. We are developing techniques for in vivo nuclear magnetic resonance imaging of grafted immature tissue.
Fetal rat (E17-E18) posterior cortex was employed in grafting studies by two methods: (a) Neonatal (P5-P7) host rats had fetal tissue fragments grafted into the posterior cortex. (b) Adult (P150) host rats received grafts as dissected cell suspensions, labeled with iron oxide particles using saline-embedded Sendai viral envelopes. NMR imaging of the adult and neonatal hosts was performed with sequential magnetic resonance images at 120 respectively. Histochecy (Prussian blue test for iron) and immunohistochemistry (GFAP) of host brains were performed on 30μm thick sections. In the NMR, the intact perfusion-fixed neonatal host brains (axial slice thickness=700μm; pixel = 60 x 60; TE = 55ms; TR = 4s), the issue fragment grafts equivalent to the surrounding cortical structures but bright with respect to the immediately adjacent white matter. Images (axilation slice thickness=700μm; pixel = 150 x 150; TE = 30ms; TR = 46s) of the brain of adult hosts, both in vivo and after fixation, showed dark regions. Light microscopy of these regions demonstrated iron oxide positive cells of probable donor origin. The iron oxide appeared as a dark blue precipitate within soma and proximal processes, suggesting good short term survival of the donor cells. The exact location of the exogenous iron and the possible reactive response within the host brain were currently being investigated. Supported by ONR N0001489-J-1556

531.2
ULTRASTRUCTURAL CHARACTERISTICS OF FETAL SUPRA-CHIASMATIC NUCLEUS TRANSPLANTS IN LATERAL AND THIRD VENTRICLES AND IN CULTURE. U. Vaidya and R. V. Moore. Dept. of Neurology, SUNY, Stony Brook N.Y. 11794
Fetal supra-chiasmatic (SN) nuclei transplants have been shown to restore circadian rhythm in SCN lesioned hosts if the transplants are located in the third, but not the lateral ventricle. In the present study, SCN transplants were placed bilaterally, in and out of cortex examined to detect any site specific differences in development. Female Sprague-Dawley host rats received 2-3 mm3 pieces of tissue from E16 donor fetuses injected unilaterally into either lateral ventricle. Explants of the SCN from the same set of donors were placed in culture. Hosts with transplants were allowed to survive for 30 days, while cultures were examined at 2 or 4 weeks after transplantation. The quality of SCN tissue developed in the lateral ventricle. Infrequent discontinuities in the ependymal lining allowed direct contact between the host and transplant and the passage of axons. The degree of integration of the transplant to the host brain was greatest in the third ventricle. Transplants in both locations and explants in culture had VIP positive neurons, and in the neuropil myelinated and unmyelinated axons, and synapses. Blood vessels were also seen in transplants. The organization of the neuropil in cultured explants was less complex than transplants in hosts. The results suggest that SCN transplants into the lateral and third ventricle have a similar origin, union and development. The greater degree of host- transplant apposition in the third ventricle was the only notable site-related difference.

531.3
ABSORAL MEDULLA SURVEYS FOR LONG ZIME PERIODS AND PREVENT DEGENERATION OF THE SUBSTAMTA NIGRA WHEN CO-GRAFTED IN THE PNS. L.C. DEERING and R.A. TOLJUS. Department of Biomedical Sciences (Anatomy), McMaster University, Hamilton, Ontario, CANADA L8N 3J5.
Grafts of fetal substantia nigra (SN) undergo a series of degenerative changes when transplanted into the peripheral nervous system (PNS). This study was performed to determine if the adrenal medulla (AM) could prevent these changes when combined with the PNS. The AM was obtained from neonatal (E17) rats, phosphorylated (Psyn2) and non-phosphorylated (SN3-31) neurofilament protein. Single nigral grafts were shrunken, contained only a few TH positive cells and displayed abnormal patterns of Psyn2 and SN3-31 immunoreactivity. In contrast, when combined with the AM the loss of TH neurons was prevented and the AM did not show the aberrant cytoarchitectural changes. Furthermore, all AM grafts were viable after one year in the PNS, while those combined with dozens of thousands of chromaffin cells identified by expression of the TH and Psyn2 epitopes. These experiments illustrate that the PNS can support grafts of AM for up to one year and the AM can offset the degeneration of SN neurons when co-grafted under the present experimental conditions. (Supported by The Parkinson Foundation of Canada)

531.4
LONG TERM IDENTIFICATION OF TRANSPLANTED RODS. P. Gouras, J. Dar, R. Ewos, R. Lopez, H. Kieldby. Columbia University Department of Ophthalmology, 630 W 168 St New York 10032
Rat rods in mitosis (at least 50%) can be radioactively labeled by administering 3H-thymidine subcutaneously to newborn rats. At 1-3 months of age these rat rods are isolated enzymatically from the retina, mixed with dissociated retinal pigmented epithelial cells from non-labeled donors and transplanted to the subretinal space of 4-5 month old congenic albino dystrophic RCS rats. At this time the dystrophic rat has virtually no rod site. The transplant site shows the speckling of pigmented epithelium in the albino retina observable both ophthalmoscopically and histologically. Examination of the host retinas after transplantation reveals the unequivocal nuclear stain of tritiated thymidine in these transplanted rods at three months (longest time) after transplantation. Electron microscopy reveals that these rods have synapses on retinula cells and retinal axons. This system is of practical use in the study of synaptic establishment in the retina.

531.5
INFLUENCE OF AFFERENT INPUT ON SURVIVAL OF FETAL NEURAL TRANSPLANTS INTO A HOST TARGET. J.H. McLellan and A.H. Darby. Div. of Basic Medical Sciences, Memorial Univ. of Newfoundland, St. John's, NF, Canada, A1B 3V6
In Alzheimer's disease, several regions of the brain degenerate, including cortical structures such as the olfactory bulb and several afferent inputs of providing replacement neurons. One of the main depleting pathways in the brain is to provide the brain with trophic and neural transplant. In these experiments, we have been employing fetal rat (E16- 17) cholinergic and serotonergic neurons from the diagonal band and raphe nuclei, respectively, to olfactory bulb of adult rats that have been previously transplanted with different regions. The results were evaluated by immunocytochemistry and Ach histochemistry to determine the degree of integration of transplanted cells into the host brain. Cholinergic and serotonergic neurons generally exhibited good survival and integration into the host olfactory bulb. Transplanted cholinergic and serotonergic neurons on the side ipsilateral to cholinergic and serotonergic neurons on the side ipsilateral to serotonin depletion survived better than transplanted cells on the contralateral side. In addition, transplanted neurons appeared to survive less well in animals that had not previously been depleted of afferent input to the bulb. These results suggest that in the brain such as a result of afferent input depletion increased the survival of transplanted cells and the trophic effect may not be limited to the side of the brain that was previously deprived of transmitter. This work was supported by the American Health Assistance Foundation and MRC of Canada.
AUTORADIOGRAPHIC STUDY OF FETAL STRIATAL GRAFTS PLACED IN HOST STRIATUM PULSE-LABELED WITH [3H]-THYMIDINE. J.C. Liu, N.G. Amatani, and R.E. Gross. Department of Pharmacology, University of Virginia, Charlottesville, VA, U.S.A.

We have studied the incorporation of labeled cells into host striatal tissue in rats. The grafts were placed in the striatum of adult rats and pulse-labeled with [3H]-thymidine. The labeled cells were then quantified and compared to control animals. The results indicate that the labeled cells are incorporated into the host tissue and that the rate of incorporation is dependent on the duration of the pulse-labeling period. These findings suggest that fetal striatal grafts can be used to repair damaged striatal tissue and that the rate of incorporation can be controlled by the duration of the pulse-labeling period.

Previous studies have indicated that barrier properties within the neurovasculature of CNS may be altered such that exogenously administered protein such as HRP may infiltrate the neuropil. To further examine this system, non-injected rats bearing cortical or nigral transplants (10 days-1 year postoperative) were perfused with 4% paraformaldehyde and examined immunocytochemically for endogenous IgG or albumin (RSA). The distribution of anti-RSA with regard to the sources of this protein was seen to be correlated with the extent of permeability of the barrier. These studies revealed that injected proteins persisted in vesicular grafts and considerably less and variable in intraparenchymal grafts. Where the allografts appeared to be immunologically rejected, anti-RSA was prominent for months. To examine access of a neurotransmitter, TRKB (250 μl) was systemically administered for 30 minutes. Notions of ventricular grafts were injected and neurons avidly sequested to the bloodstream transmitter which normally never crosses the blood-brain barrier. The results confirm that barriers may be altered and CNS grafts can be exposed to endogenous proteins administered neurotransmitters such as GABA (NS-17668).

CONCENTRATION DEPENDENCE OF PHOTORECEPTOR CELL RESCUE BY RPE TRANSPLANTS. L. Li and J.E. Turner Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

We have reported previously that photoreceptor cells are rescued by retinal pigment epithelial (RPE) cell transplants in RCS dystrophic rats (Li and Turner, Exp. Eye Res., 1988). Further studies showed that healthy RPE cells were required for the long-term rescue including outer segment integrity. However, vehicle injections initiated a transient, local beneficial effect which diminished with time. In this study, different concentrations of RPE cells were injected into the subretinal space of RCS rats. When analyzed at 1 and 2.5 months after transplantation photoreceptor cell rescue was seen in all groups. However, the results revealed a concentration dependence and saturation of this effect. Therefore, we conclude that healthy RPE cells are required for long term rescue of photoreceptor cells and this rescue is concentration dependent and saturable which is not the case with vehicle injections. This research was supported by grants from NIH (EY04377–08), the National Retinitis Pigmentosa Foundation and Retinitis Pigmentosa Intl.

DEVELOPMENT AND PLASTICITY—VISUAL SYSTEM: RETINOTECTAL CONNECTIONS

DEVELOPMENT OF THE RETINOTECTAL PROJECTION OF NASO-VENTRAL QUARTER EYES IN XENOPUS LAEVIS. N. Degen, L. Peter, and K. Brindke.

Zool Inst, Univ. Frankfurt, Stieglereydt 70, D-6000 Frankfurt 11, West Germany.

According to Speransky's chemotactic hypothesis (Speransky, J. Proc Natl Acad Sci USA 50:141, 1963) the projection of a small eye fragment with a reduced amount of optic fibers should be restricted to that position in the tectum corresponding to its own specificity. In order to verify this hypothesis we removed three quarters of the eye anlagen in Xenopus embryos of tail bud stages. The resulting quadrant of the tectum was observed until the end of metamorphosis. We found that the neural retina still projected to the contralateral part of the tectum. Since in normal Xenopus eyes some fibers seem to retain their original specificity (Degen, N. and Brindke, K. Acta Biol Hung 39:191, 1988) it can be excluded by the results that fibers of a quarter eye initially occupied an area corresponding to their own specificity. Electrophysiological recordings showed that the retinae of Xenopus eyes are topographically organized. This supports a selfassembly of ingrowing optic fibers of more than the preexistent markers in a virgin tectum. This supports the initial establishment of the retinotectal map.

ACTIVITY DEPENDENT AND INDEPENDENT ASPECTS OF TOPOGRAPIC MAP FORMATION IN MAMMALS. D.K. Simon and D.D.M. O'Leary.

Departments of Neurosurgery and of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri.

Retinal axons form a highly ordered, topographic map in the superior colliculus (SC) of adult rats. However, developing retinal axons mislabel widely in the SC, resulting in an initially diffuse retinocollicular projection (O'Leary et al. 1986 J Neurosci 6:3092; Simon & O'Leary 1990 Dev Biol 137:125). Normally, most mislabeled axons are eliminated by P12. Retrograde labeling suggests that major targeting errors are removed even when retinal activity is blocked (O'Leary et al. 1986). Here we use DII as an anterograde tracer to examine activity-dependent and independent aspects of retinotectal connections. We label the ganglion cell bodies in the retina that grow into the appropriate topographic position in rostral SC. This observation indicates that even in the absence of retinal activity many axons are able to arborize at the topographically appropriate region. Therefore, the formation of a topographically appropriate arbor can be governed by activity independent mechanisms. In contrast, the elimination of abnormally positioned axons and branches is to some extent activity dependent since their removal is deterred (or delayed) by blocking retinal activity.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

During the first week after birth, the neonatal retinal system undergoes several complex processes, e.g., synaptogenesis and cell death. We investigated whether action potentials or synaptic transmission could play a role in these processes by measuring spontaneous and evoked responses in the developing retinotectal pathway. In preliminary experiments, we studied 16 rat pups from postnatal day 6 (P6) to P14. Glass microelectrodes (NaCl, 1-2 Mohns) were lowered through the superficial superior colliculus while measuring single unit activity or optic nerve activity. On P6/P7, spontaneous activity was rare (n = 3 cases). Starting on P8, there was spontaneous activity in collicular neurons (n = 13 cases). Action potentials were measured in response to electrical stimulation of the contralateral optic nerve. From P6-P9 (n = 7), evoked responses were inconsistent, with latencies of about 35 msec. However, from P10-P14 (n = 7), there were action potentials to optic nerve stimulation. The responses were spikes with a latency of about 15 msec and a stimulus threshold of 60 V. Both spontaneous and evoked responses were biphasic, indicating a somatic origin. In a previous abstract (Soc. Neurosci., 1988), we reported that flash evoked responses first appear around P12/13. Our results suggest that the retinotectal pathway becomes functionally capable in three stages: (1) spontaneous activity first appears on P8; (2) electrically evoked collicular responses develop on P10; and (3) flash-evoked responses appear on P12/13. This suggests that the retinotectal pathway becomes functional during the second week after birth.

PROTEIN COMPONENTS OF THE RETINOTECTAL MAPPING MECHANISM. F. Steen and M. Constantine-Paton, Biology Dept, Yale Univ., New Haven, CT 06511

The NMDA receptor is an integral component of the activity dependent retinotectal mapping mechanism. In the Xenopus laevis retina, the NMDA receptor antagonist, APV, disrupts the precision of this map (Cline and Constantine-Paton, Neuro 3:143, 1989) and disrupts eye-specific segregation in three eyes from Xenopus retina (NMDA) striate sharpening this segregation (Cline et al., PNAS, 84:432, 1987). It is our objective to identify other molecules associated with this machinery. Since activation of the NMDA receptor appears to be the first step in a cascade of events, we have chronically treated animals with APV and NMDA (10\(^{-6}\)M in ELVAX implants for 4.5 weeks) and examined these proteins for reversal of treatment. We have previously reported results of two-dimensional gel analysis after such treatments (Steen and Constantine-Paton, Soc. Neurosci. Abstr. 1:121, 1990). However, since high molecular weight proteoses do not focus under SDS-PAGE, we have not been able to examine these analyses. Therefore, we have utilized silver stained SDS-PAGE to identify any changes that occurred during our treatment. We have identified two proteins of approximately 240 and 175 kD which increase in abundance following chronic APV treatment, a 185 kD protein which decreases in abundance after APV treatment, and a 150 kD protein which decreases in abundance following chronic NMDA treatment. The 185 kD protein also appears to migrate slightly slower in the APV treated animals, with an increased apparent molecular weight of about 5 kD. We suggest that not only the changes in these proteins may coin the changes in the retinotectal map, but that these proteins are integral components of the mechanism which forms and maintains this map. Supported by NIH grant EY00639.


Cytochrome oxidase (CO) expression in some visual centers has been shown to be correlated with retinal activity levels (Wong-Riley, 1989). Moreover, development of transient CO activity in some visual areas has been correlated with retinal activity (Crossland and Peduzzi, 1987). The CO activity in the tectum appears to be embryonic in origin, and the mature form of the CO activity closely linked to the arrival of retinal axons or is it an independent cell property (Lazich et al., 1987) correlated with tectal neuronal birthdate. We have investigated this question in the optic tectum of the embryonic chick. The order of tectal laminar neurogenesis in the chick (Lavall and Cowan, 1971) is: 1. Laminar Structures (SGS), 2. Stratum griseum et fibrorum (SGFR), and 3. Stratum griseum et parvicellularum (SGP). Stratum griseum et parvicellularum (SGFr) (Sten and pharmacological chemical methods (Wong-Riley, 1979) were used on reo-filling frozen sections of aldehyde-fixed brains from E9 to adult chickens. On E9, when the tectum is at stage 12, CO activity was highest in SGS, SGP and SGr, and a band within the pretectal region. By E10, the SGS and SGr were inviolated to the innervated retinal tectal regions even though CO reactive retinal ganglion cells were evident at this stage. The role of visual input in the pattern of CO activity is further explored in the companion study. On E14, the CO activity was highest in stage 11 whole mount preparations. The amount of CO reactive blood vessels innervated in the retinal tectum was measured over the duration of the study. The introduction of new CO reactive bands and their further laminar differentiation in the SGS and SGr was measured at E14, which coincides with the retinal CO reactive retinal ganglion cells were evident at this stage. The role of visual input in the pattern of CO activity is further explored in the companion study. On E14, the CO activity was highest in stage 11 whole mount preparations. The amount of CO reactive blood vessels innervated in the retinal tectum was measured over the duration of the study. The role of visual input in the pattern of CO activity is further explored in the companion study.
532.9


Monoclonal antibodies made against goldfish tectal tissue labeled specific cell types in the brain. In the tectum, the pyramidal neurons (Type I) whose soma are located in the SPFUs were intensely labeled with mAb 2F8. Purkinje neurons in the corpus cerebelli and valvula cerebelli and the oculomotor neurons were also labeled. In addition to the neurons, labeled glia types include: a) Muller cells in the retina, b) oligodendrocyte-like cells in the optic nerve and c) radial glia in the tectum. No labeled glia were observed in other regions of the brain. During development the antigen recognized by mAb 2F8 appeared first on the Muller cells of the retina before hatching and by 11-14 days post hatching within the cells of the tectum, cerebellum and oculomotor nuclei. At present, it is not clear whether the antigen recognized by mAb 2F8 is the same in the different cell types or a single epitope is shared by several antigens. This antibody will be useful for studying the response of tectal and Purkinje neurons to injury and will aid in the isolation of the different cell types that are a tissue culture system. Supported by NEI 1426.

532.10


Eighteen day embryonic rat eyes (E18) were either sutured to a 3 cm segment of adult rat sciatric nerve or attached with purified gelled cyanoacrylate. In animals that provided the sciatric nerve segment, the left optic nerve was completed transsected 2 mm distal to the orbit and the lens removed. The E18 eye/sciatric nerve bridge was then implanted into the host's eye, while the proximal end of the sciatric nerve bridge was inserted through a burr hole in the cranium into the contralateral superior colliculus.

One year post-implantation of the fetal eye/sciatric nerve bridge, a 20% solution of HRP was injected intraocularly. Twenty-four to forty-eight hours post injection, the animals were sacrificed and the fetal eye bridge and brain processed for HRP histochrome or electron microscopy. At the light microscopic level, HRP was observed in the bridge and superior colliculus while at the EM level, labelled myelinated axons in the bridge and synapses in the superior colliculus were observed. Supported by NIH RO1 NS19245.

532.11

EARLY MONOCULAR ENucleATION AND GENICULOCORTICAL TOPOGRAPHY IN THE GOLDEN HAMSTER. A.J.Trevesyan* and J.D.Thompson* (SPON: Brain Research Association). University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, United Kingdom.

We have used a double-label technique to study mapping precision in geniculocortical-projection of normal adult hamsters and those enucleated at neonatal ages. Green and rhodamine fluorescent latex microspheres (Katz and Iaroci, 1990, Neurosci., 34, 511-520) were used as the retrograde markers. Small, discrete injections (150nl) of each marker were made (through glass micropipettes) into area 17 on both sides, contralateral and ipsilateral to the remaining eye. The labelling in the dorsal-lateral geniculate nucleus (dLGN) was studied in serial brain sections made by UHR microscopy. The number of cells labelled from a single injection was very similar in both normal and enucleated nuclei. This suggests that the number of geniculate cells projecting after birth is the same.

In the enucleated animal, the dLGN still receiving retinal input has an essentially normal pattern of projection. However, analysis of the distributions of double-labelled cells and of the overlap in the two populations of labelled cells in the dLGN shows that early removal of retinal input perturbs the development of precision in geniculocortical topography.

532.12


Little is known about the development of topographic organization between thalamic projections and their cortical target. In the hamster, the lateral geniculate nucleus is produced between E9.5 and E12.5 (birth is E16). Their axons do not invade the cortical target area until three days after birth. We examined the development of these projections and their interactions with developing cortex. Di was placed in the dorsal thalamus of fixed brains of E14 and E16 hamster pups. After two months the brains were sectioned with a vibratome at 75-100um and examined with fluorescent microscopy.

The growing axons are fasciculated in part of the internal capsule but do not maintain nearest neighborhood relations. Fascicles branch and cross, and fibers leave one fascicle to join another. In E14 animals thalamic fibers have grown through the internal capsule, but only a few extend beyond this to traverse a short distance under the developing cortical plate. No retrogradely labelled cell bodies are seen in the region of the cortical plate in E14 animals. In E16 animals thalamic fibers have grown beyond the internal capsule and extend well into dorsal regions of the developing cortex. Retrogradely labelled cell bodies are seen scattered within the growing fibers and more superficially in the developing cortical plate. These subplate projections do not reach the thalamus until approximately the same time that the thalamic projections reach the cortex. Supported by NIH RO1 NS19245.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
533.1 DONOR CLIMBING FIBERS SYNAPSE ON HOST PURINIC SPINES  
K. Kawamura, S. Murase, T. Yusa and K. Yoshioka 

In the host rat cerebellum, where the inferior olive and climbing fibers had been destroyed by intratentorial injection of 3-β-sodium selenite(3-β-SeO3), the medullary spinal tractum from (E3-E16) containing the inferior olive was grafted. After 3 weeks, climbing fiber type terminals bearing densely-packed round vesicles were found that established synaptic contacts on dendritic spines of the host Purkinje cells. Quantitative analysis at the ultrastructural level reveals that the control was taken from 3-4F treated, non-grafted site. Main results are the followings. 1) The number of typical climbing fiber terminals increased from 0.35 to 2.6 f/g after grafting, which was statistically significant (p=0.01). 2) The number of typical parallel fiber type terminals that contained spongiformly distributed round vesicles increased from 13% to 27% after grafting, which was also statistically significant (p=0.01). It is considered that competition of searching targets is likely to occur between the donor climbing and the host parallel fibers in the process of re-modelling the brain.

533.2 A QUANTITATIVE ELECTRON MICROSCOPIC STUDY OF SYNAPSE FORMATION BETWEEN CULTURED CEREBRAL CORTEXAL NEURONS.  
Masami Ichikawa, Kazuyo Muramoto, Kazuo Kobayashi1 and Yoichi Kurihara  
Embryo., and Neurochem., Tokyo Metropolitan Inst. for Neuroscience, Fuchu, Tokyo 183, Japan.

Monitoring of the formation of synapses among dissociated cerebral cortical neurons in vitro using video-assisted multi-site Ca2+ fluorometry has shown that the presence of the eco-protein kinase inhibitor (K-252b) inhibits synapse formation (Muramoto et al., 1988). We have quantitatively the effect of K-252b on synapse formation using electron microscopy. Cerebral cortical cells of rat embryo (18 days) were cultured. After 7 days, the cultured cells were fixed and embedded. Ultrathin sections were cut and photographed randomly, and synaptic contacts were counted on the electron micrographs. The examined area covered more than 3800 μm2 per culture. The density of synapses was 1.35 ± 0.23/150 μm2 in control. In the presence of 0.8 μM K-252b, the density of synapses was decreased to 0.58 ± 0.23. This result shows that synapse formation is inhibited by K-252b and confirms the result of the functional assay using fluorometry. These data support the idea that K-252b phosphorylates surface proteins involved in synapse formation.

533.3 ONTOGENY OF CHOLINE UPTAKE SITES AND M1 MUSCARINIC RECEPTORS IN THE BABOON HIPPOCAMPAL FORMATION 
Hei Hahn, Harvey S. Singer, Larry Walker, Pedro Lewenstein, Ronald Silverman, Donald Price, Joseph Cole 
Johns Hopkins Univ Sch of Med, Baltimore, MD 21205.

The developmental maturation of the cholinergic septohippocampal system in baboon hippocampal (HF) was analyzed using quantitative receptor autoradiography. On frozen sections, from midgestation, newborn, juvenile, and young baboons, we used [3H]-N-ethyl-N-(2-pyrindyl)pirazoline (N-PIR) to label the cholinergic uptake site and [3H]-pirenzepine ([3H]-PZ) to label M1 muscarinic receptors present in CA1, dentate gyrus DG, and subiculum (SUB) at 100 days gestation, with highest densities in CA1 and CA2. Total [3H]-PZ binding increased two- to three-fold between birth in all regions except SUB, then declined by adolescence. Between ages 3 to 5 years, densities were stable in CA1, DG, and SUB. [3H]-HC3 binding, after only modest increases from midgestational levels, tended to peak at birth with only slight alterations into adulthood. [3H]-PIR was always greater than [3H]-HC3 binding. Density in CA1 and CA2 was most intense over the pyramidal layer; in DG, the molecular layer exceeded the granular layer. In baboon HF, although cholinergic uptake site carriers and postsynaptic receptors are present in an adult distribution by midgestation, binding densities have different developmental patterns.

533.4 AN ANALYSIS OF RECURRENT EPPS RECORDED IN IMMATURE CA3 HIPPOCAMPAL NEURONS, J.V. Swann, and K.L. Smith  
Wadsworth Center for Labs and Research, NY State Dept. of Health, Albany, NY.

Excitatory synaptic interactions between pairs of CA3 hippocampal neurons were studied in vitro slices of hippocampi taken from rats 10-16 days of age. Experiments were performed in the presence of penicillin in order to suppress synaptic inhibition and permit the full expression of the local excitatory synaptic interactions. In 4 of 14 pairs of cells monosynaptic interactions were recorded. The unitary epps were unusually large and prolonged. At midgestation, epps were of between -60 and -70 the average amplitude was 7.28 ± 9.44 mV. Epps were usually more than 200 msec in duration. The probability of an epp was also high, 0.94 ± 0.14. The decay of the epp was much slower than that of responses to somatically injected current. Typically the recurrent epps were not followed by an afterhyperpolarization or undershoot as has been reported in CA3 pyramidal cells from mature animals. This would suggest that the k+ conductance that has been reported to underlie such undershoots has yet to appear at this stage in hippocampal development. Presynaptic burst firing results in dramatic facilitation of unitary epps. Our results suggest that, while recurrent excitatory synapses are present early in postnatal life, the epps they produce differ in many ways from their counterparts of adulthood. Support NS18309.

533.5 MORPHOLOGICAL STUDIES OF CA3 HIPPOCAMPAL NEURONS IN THE DEVELOPING RAT. C.M. Gomez, F.J. Bice, K.J. Smith* 

We are studying the morphological substrates which may be related to an increased propensity the immature CA3 hippocampal neurons from animals of different ages were characterized physiologically in slice preparation. Intracellularly and biocytin. Preliminary morphometric analysis of serially reconstructed filled pyramidal cells revealed that the most extensive axonal arborizations occur in the second postnatal week as compared to the first or to adulthood. In week two, we observed the most elaborate axonal networks with variations in aspect (likely sites of synaptic contact) in Stratum oriens (S. oriens). Several S. oriens and S. radiatum but few were seen in the cell body layer. Neurons from the first postnatal week had more restricted processes. Adult neurons had a dramatic reduction in the axonal networks of S. oriens. Some unique cells had cell-like synaptic characteristics but basket cell-like axonal plaxi in the cell body layer and unusual fine axons in S. oriens and radiatum. We postulate that a developmental process of synapse loss results which results in a surplus of excitatory contacts and enhanced seizure susceptibility in week two. Excess synapses regress with maturation, imparting decreased propensity for seizure induction.

533.6 ONTOGENY OF DOPAMINERGIC AND GABAERGIC INNERRATION OF THE INTERMEDIATE LOBE OF THE RAT PITUITARY GLAND. K.A. Cary and B.M. Chronwall  
School of Basic Life Sciences, University of Missouri-Kansas City, Kansas City, MO 64108.

Dopamine and GABA are co-localized in axons innervating melanotropes of the intermediate lobe (IL) of adult rat pituitary. Transmitter localization in the IL may not appear at the same time during development. To determine the sequence and time of innervation, we stained Sprague-Dawley IIs at embryo days (E) 17-20 and post-natal days (PN) 3, 5, 10, and 26 immunohistochemically using dopamine, tyrosine hydroxylase (TH), GABA, and a glutamine deacetylase (GAD) antiserum. TH and GAD localization on sections identified regional axonal populations prior to the final pattern of innervation in the IL. Demonstration of the synthesizing enzyme and the final neurotransmitter is important to show neuronal synthesis rather than axonal uptake of the neurotransmitter. The final pattern of innervation was observed in E17 through PN 2. At PN 3, TH, dopamine and GABA immunoreactivities were all demonstrated. GABA distribution at PN 3 was very similar to that in the adult. Conversely, TH and dopamine concentrations at PN 5 are highest in the caudal aspect of the IL, and gradually decline rostrally. By PN 2B, the spatial distribution of TH and dopamine is similar to that observed in the adult but staining intensity is much less than seen in adult IL. These results indicate a possible difference in onset of dopaminergic and GABAergic innervation occurring in the IL during ontogeny.
33.3.7 PSYNAPTIC DEVELOPMENT OF MONOAMINERGIC RECEPTORS IN THE FEMALE NEOCORTEX. M.S. Lidow, P.S. Goldman-Rakic, D.W. Gallagher, and T.R. Jacobson. Section of Neuroanatomy, Tulane University. School of Medicine, New Orleans. CI 00510.

Quantitative in vitro autoradiography was used to determine the postnatal development of D1 and D2 dopaminergic, α2 and β norepinepheric, and 5-HT1A serotoninergic receptors in fetal, prefrontal, somatosensory, parietal, and visual cortex of rhesus monkey. At least two animals have been examined at birth, 1, 2, 4, 8, 12, 36 and 60 months of age. We found that the density of the various receptor subtypes exhibits a similar course of postnatal changes which take place simultaneously throughout all cortical areas examined. The density of receptor binding is quantitated by a computerized method which assumes that the density of receptor binding is linearly related to the number of receptors per unit area. Moreover, the density of binding sites in the fetal and newborn rabbit thalamus, retina, and cerebellum was found to be in agreement with previous autoradiographic studies.


The present study was undertaken to identify potential molecular markers that may characterize the cellular mechanisms that promote cell survival and induce synaptic remodeling following neuronal deafferentation. For this study, we used an established rat deafferentation model in which the thalamo-cortical corticospinal fibers are induced to sprout and innervate deafferented striatal target neurons following a unilateral cortical lesion. We have identified a gene whose expression is upregulated in striatal deafferented neurons and protein content for glial fibrillary acidic protein (GFAP), an established marker for reactive astroglia and sulfated glycoprotein SGP-2 (SGP-2), a putative inhibitor of complement-dependent cytotoxicity.

We found that mRNA prevalence for both GFAP and SGP-2 in the ipsilateral ST was increased at 3 days postlesion and reached a maximum (5-10 fold) at 10 days postlesion. By 27 days postlesion GFAP and SGP-2 mRNA prevalence were reduced but still elevated over levels found in the intact ST. Changes in mRNA prevalence for GFAP and SGP-2 were correlated with an increase in the density of immunoreactive fibers for both markers in the ipsilateral ST and paralleled the time course required for the homotypic reorganization of the lesioned ST by axonal fibers from the contralateral cortex. We hypothesize that both GFAP and SGP-2 play important roles in the cellular events associated with the phenomenon of reactive synaptogenesis following neuronal deafferentation and that SGP-2 serves to protect striatal neurons from phagocytic attack while reactive astrocytes remove surrounding degenerative axon and dendritic profiles from the lesioned cortex.

33.3.9 ACTIVITY-DEPENDENT RELEASE OF ATP ACTIVATES ECTO-PROTEIN KINASE IN TO SIMULATE SYNAPSE FORMATION BETWEEN CULTURED CEREBRAL NEURONS. Yoichiro Kuroda, Kazuyu Muramoto, Kazuo Kobayashi, Masumi Ichiwaka. Dept. of Neurochemistry, National Institute of Medical Research, Bldg. 7, Fuchu-shi, Tokyo, Japan.

Presynaptic breakdown of ATP and its breakdown products, adenosine derivatives inhibit and facilitate synaptic transmission in the CNS and have been proposed as the main mediators of activity-dependent synaptic competition for binding sites in the human long-term memory. Our hypothesis is that ATP releases (K-252b) inhibits the synaptic formation in vivo (Muramoto et al. Proc. Jpn. Acad. Ser. B, 65, 319, 1989). Extracellular addition of ATP induced phosphorylation of several cell surface proteins in the culture. To demonstrate the activity-dependent changes in synaptic contacts, we cultured dissociated cerebral cortical neurons with various concentration of ATP for 7 days. ATP significantly stimulated the synaps formation as observed quantitatively by electron microscopy. These data indicate that the activity-dependent release of ATP provides substrate for the ecto-protein kinase to phosphorylate functional proteins on the synaptic membranes, which stimulate sprouting and synapse formation. This could selectively stabilize the "tracing circuits" for human long-term memory.


Synaptophysin (GP39) is the most abundant neurotransmitter vesicle. We have characterized a glycoprotein with an apparent molecular weight of 39 kD, designated GP39, from chick brain using a monoclonal antibody. Purification of the protein from chick forebrain by immunofinity chromatography and N-terminal sequence analysis showed GP39 is a homologue of mammalian synaptophysin.

Our immunohistochemical results indicate that similarly to synaptophysin, GP39 was present in virtually all neurovascular terminals in adenial and basal ganglia cells. The pattern of immunostaining of the brain, spinal cord, retina, and neomuscular junction was consistent with that reported for synaptophysin and supports the belief that this is an abundant component of small synaptic vesicle pools of the neurotransmitter involved. Solubilization experiments confirmed GP39 was an integral membrane component without any tight cytoskeletal associations. It was N-glycosylated and was demonstrated to produce a 34 kD form with N-Glycanase treatment. The glycoprotein was highly susceptible to proteolysis with a tendency to form a 23-25 kD fragment.

The deduced amino acid sequences of mammalian synaptophysin indicate an evolutionarily highly conserved molecule with four transmembrane spanning regions and a novel cytoplasmic domain. Such a structure has been proposed to imply a possible function as an ion channel with the carboxyl cytoplasmic domain perhaps serving as an extracellular binding site (Sudhof, T.C. et al., Science 238:1142-1144, 1987). Alternatively, it has been suggested to function in calcium-mediated neurotransmitter release. Our studies of the avian molecule also define its role in the development and function of the synaptic terminal.

ONTOGENY OF NEUROENDOCRINE SYSTEMS


GAP-43 is a neuronal phosphoprotein associated with axonal growth during development, plasticity and regeneration. Studies in various species have shown that both GAP-43 mRNA and protein are expressed at high levels early during postnatal development and decrease precipitously during the course of maturation. The aim of the present experiments was to closely compare mRNA expression (as visualized by in situ hybridization) and protein expression of the GAP-43 protein (visualized by immunocytochemistry) (IC) in several forebrain regions during postnatal development of the BALB/CByJ mouse. In addition, we examined neonatal lesions among the cortically projecting, cholinergic basal forebrain neurons (nMB) to determine whether changes in the time course of cortical morphogenesis would alter GAP-43 expression. Indeed, nMB lesions have previously been shown to result in a retardation of cortical neuronal differentiation.

Our results indicate that expression of both mRNA and IC for GAP-43 is highest during the first week postnatal, decreased by 2 weeks and is further reduced by one month. However, incongruencies between mRNA expression and IC localization became apparent as a function of distribution and relative levels between different regions of the forebrain. For example, mRNA expression was higher in cortex and hippocampus than in the striatum at all ages while immunoreactivity appeared higher in the striatum, particularly in adulthood. On the other hand, the ventrobasal complex of the thalamus did not express high levels of either mRNA or protein throughout development. Moreover, quantitative data indicate that GAP-43 expression in cortical pyramidal neurons as late as 1 year postnatal. Finally, neonatal nMB lesions appear to subtly affect the time course of GAP-43 IR in cell bodies of the ipsilateral cortex and hippocampus.

334.2 NPY Gene Expression in Developing Mouse Brain: An In situ Hybridization Study. G. Capone, C. Homanick, and J.T. Coyle. Johns Hopkins School of Medicine, Dept. of Pediatrics, Psychiatry and Behavioral Sciences; The Johns Hopkins University School of Medicine, Baltimore, MD.

Previous immunocytochemical studies have shown "NPY like" immunoreactivity throughout brain regions during development and adulthood. We have used an in situ hybridization approach to regional distribution of NPY mRNA in the brains of Balb/CByJ mice at various postnatal ages (in utero to adulthood). A specific, clearly discernable ISH signal was seen overlying discrete cell bodies within the cortex, hippocampus, caudate-putamen, claustrum, olfactory tubercle, endopiriform nuclei, and NPY immunoreactive terminals in the dorsal thalamic nuclei. On PND 7, signal intensity was highest in cells from the thalamus, caudate-putamen, and hippocampus, and low throughout most of the cerebral cortex. By PND 14, signal intensity increased in the cortex, remained about the same in hippocampus and caudate-putamen, and decreased slightly in thalamus. In the cerebral cortex and several other brain regions, there is an apparent increase in the number and intensity of NPY expressing cells during the first several weeks of postnatal life, relative to the adult. The regional anatomic distribution of NPY mRNA expression in the developing mouse brain is discussed.

We have demonstrated a transient increase in the number of somatostatin (Smst)-expressing cells in the cerebral cortex following neonatal nMB lesions. Because NPY and Smst are co-localized in many of the same cortical neurons, we are examining NPY gene expression in the cerebral cortex following nMB lesions.
534.3

Angiotensin is best known for its role in blood pressure and electrolyte balance. The present study reports that angiotensin may also have a role in growth and development. Angiotensin can stimulate cell growth, increase release of growth factors, and stimulate expression of growth-related proto-oncogenes. Therefore, the localization of angiotensin in the brain during early development would be a logical first step in the identification of a possible role in the CNS. Of additional interest would be any differential angiotensin growth-related patterns between the spontaneously hypertensive rat (SHR) and the normotensive rat (WKY). It is suggested that angiotensin receptor binding sites could be indicative of a hypertensive pathogenesis. This study, therefore, utilizes receptor autoradiography to show the localization of 125I-Sar1, Ile8-Ang II receptor binding in the brains of SHR and WKY rats during perinatal development. Specific receptor binding sites were localized and quantified in several angiotensin-related areas of both SHR and WKY rat brains.

534.4

The effects of thyroid hormone treatment on the development of CHAT immunostained cortical fibers in the cerebral corona were evaluated. Rat pups were made hyperthyroid by administering 1 mg/kg triiodothyronine (T3) i.p. daily, or hyperhydroid by providing 0.3% propylthiouracil in the diet of the dams. Pups were sacrificed at 1, 2, 3, and 4 postnatal weeks. Brain sections were immunostained for CHAT and fiber densities, within the frontal, parietal, temporal, occipital, perineural, and retrosplenial cortices as well as the hippocampus, dentate gyrus, and basolateral amygdala were compared between the groups. In the 1 week postnatal brain, significant differences were observed for all groups. At 2 weeks postnatally, more CHAT fibers were present than at 1 week, however, fiber density analysis showed no differences between the hyperthyroid, control, and hypothyroid rat brains. Levels of CHAT fiber staining density increased in the 3-week animals but no further increases were detected in 4 week postnatal brains. At both 3 and 4 weeks, the optical density of CHAT fibers in cortical areas were greater in the T3 treated pups than both control pups (p < 0.05) and hypothyroid pups were less dense than those in control pups (p < 0.05). [Support: USPHS grant NS 10928 to L.L.B.]

534.5

We have studied the development of GABA neurons in the rat striatum by measuring levels of GAD mRNA by in situ hybridization, Northern blot analysis, and enzymatic assays. Two genes encode two molecular forms of GAD, GAD67 and GAD65, which differ in their dependence on pyridoxal phosphate (PLP). VIP receptor activation is mediated by a PLP-dependent enzymatic activity in striatum homogenates, which includes the subunit of the GABAergic neurotransmitter system. The selective increase in GAD67 mRNA is associated with the innervation of the SN by GABAergic neurons of the striatal matrix, which innervate the SN from F3 to F7. The increased expression of GAD67 is responsible for the increase in PLP-dependent activity in striatal homogenates. The selective increase in GAD67 mRNA may be responsible for increased susceptibility to PLP-antagonist induced seizures after F7. [Supported by NS08713 (RGC) and NS22256 (AJT)].

534.6

The findings made by the authors who have studied the development of GABA neurons in the striatum indicate that the increase in GAD mRNA levels may be responsible for the increase in PLP-dependent activity in striatum homogenates. The selective increase in GAD67 mRNA is associated with the innervation of the SN by GABAergic neurons of the striatal matrix, which innervate the SN from F3 to F7. The increased expression of GAD67 is responsible for the increase in PLP-dependent activity in striatal homogenates. The selective increase in GAD67 mRNA may be responsible for increased susceptibility to PLP-antagonist induced seizures after F7. [Supported by NS08713 (RGC) and NS22256 (AJT)].

534.7
DEVELOPMENTAL REGULATION OF ENDOThElIN GENE EXPRESSION IN THE HUMAN BRAIN. Alan R. Hall1, Michael B. Conway1, Stuart D. Cook2, Lynne H. Parker-Behlen1, and Christiane Cede3.

1Torrey Research Laboratory, UMDNJ-Newark, NJ 07103
2Department of Neurosciences, UMDNJ-Newark, NJ 07103
3Department of Biochemistry, Cede Laboratories, Inc., Cedar Knolls, NJ 07927, U.S.A.

The endothelins comprise a family of small, (21-30 amino acid) structurally related polypeptides, that exhibit potent vasoconstrictive activity. Other, non-vascular tissues, including the brain, also contain immunoreactive endothelin. We used radioimmunoassays for endothelin (ET)-1 and ET-3 to measure these two isoforms in human brain homogenates at various stages of normal neurogenesis. Low, but detectable (<200 fmol/mg protein) of both proteins were present in fetal brain as early as 47 days post-conception. Neural ET-1 and ET-3 levels increased during gestation and were maximally expressed in the neonatal/adult brain. These findings suggest that the endothelin gene(s) are regulated during human neuroembryogenesis.

(Supported in part by NIH/NCI grant CA49422-01 to A.R.Hall)

534.8

Mature male rats received bilateral electrolytic lesions (L) in the lateral hypothalamic area (LHA). One group of sham-operated controls was fed ad libitum (CON-ADLIB), a second CON group was pair-fed to the LHA rats (CON-FF). Two days later all rats were killed by decapitation. Body weight, food intake (FI), food efficiency, carcass fat, liver weight, epididymal fat pad (PAD) weight, incorporation of glucose-U-C14 carbon (GLUCING) into liver, total lipid and glycerol, plasma glucose and insulin were significantly (SIG) reduced in LHA vs. CON-ADLIB. Carcass protein, PAD protein and GLUCING into PAD lipid and glycerol were seen in LHA rats vs. PAD total lipid and plasma free fatty acids (FFA) were higher in LHA vs. CON-ADLIB. Compared to CON-ADLIB, CON-PP showed the identical pattern seen in LHA rats. PAD weight was comparable to CON-ADLIB and that CON-PP had less SIG GLUCING into PAD glycogen than CON-ADLIB. Furthermore, CON-PP had lower plasma glucose GLUCING into liver glycogen and PAD total lipid and lower carcass protein than LHA rats. Plasma FFA were higher in CON-PP than in LHA rats. Some metabolic changes in LHA rats appear sooner after 48 h and appear to be food-related. Other changes appear to be independent of reduced FI. Supported by VA and Baylor Coll. Dent. Research funds.
353.1

PRODOMPHIN mRNA REGULATION BY GABA A RECEPTOR IN THE MOUSE STRIATUM. C. Jomary*, J.O. Schwartz*, and C. Lorenz- Deprez*. Johns Hopkins University, Medical School, Molecular Neurobiology Unit, NIDA/NIH, P.O. Box 2180, Baltimore, MD21224 USA. Unité de Neurobiologie et Pharmacologie (U109) de l'INSERM, Geneva Paul Broca, 2 ter rue d'Alesia, 75014 Paris, FRANCE.

There is good evidence that striatal GABA receptors control enkephalin levels, release in vitro and in vivo and biosynthesis. Early studies have shown that GABA-A receptor stimulation induced a decrease in striatal enkephalin release and an inhibition of enkephalin gene expression in mouse striatum. In order to know if GABA receptors also modulate dynorphin biosynthesis, we have investigated the effect of GABAergic agents on dynorphin gene expression in mouse striatum. An acute or chronic i.v. administration of muscimol (GABA-A receptor agonist) plus diazepam (benzodiazepine receptor agonist), which potentiates the effect of muscimol, has been tested. Northern hybridization studies, done on half of the same animals treated, supported these observations. These results suggest that muscimol and diazepam have a similar effect on acute treatment. On the other hand, the acute induction of preprodynorphin RNA in the striatum, that in this case, the GABA system may regulate the expression of the protein gene in a different manner.

353.2


Two major GAD transcripts are present in developing mouse brain (3.7kb and 5.7kb) as early as day 11. A 5.7kb message is predominant in embryo and virtually undetectable after birth. The quantification of the 3.7kb transcript increases only by 50% and reaches its adult level by birth. On the other hand the adult form of human GAD67 (59 kDa and 62 kDa) are hardly detectable until the end of the first postnatal week. At the same time a 40 kDa protein can be detected by immunostaining of Western blots of brain extracts from ages E13 to P14. Recently we have identified 2 GAD genomic loci in the mouse Gad-1 and Gad-1ps on Chr 2 and respectively. Gad-1ps by many criteria resembles a processed pseudogene which has a moderately and highly homologous region with mouse Gad CDNA separated by an inframe stop codon. This indicates that Gad-1ps might have arisen from a different transcript expressed very early in development. A MET following the stop codon could serve as a very strong potential initiation site for the translation of the rest of the ORF coding for a 45 kDa protein which is in good agreement with the size of the embryonic form of GAD. Based on our results we propose that a transcript different from the adult one codes for a truncated embryonic GAD protein.

353.3


Haloperidol and other antipsychotic drugs require 1-2 weeks before they ameliorate some of the symptoms of schizophrenia. Rats treated with these drugs develop increased numbers of D2 dopamine receptors. The mechanism involved in this receptor up-regulation is unknown but may result from: a) an increased synthesis rate due to elevated levels of state RNA (i.e. I, b) reduced degradation. Our results suggest that neither of these explanations are in play. New work shows that the levels of the D2 receptor mRNA can change in the striatum of animals treated with haloperidol. These changes differ by as much as 10 fold from different species treated with the drug. The results suggest that the D2 receptor is not the only dopaminergic receptor affected by haloperidol treatment and that other systems, not involving the dopaminergic system, may be involved.

353.4

ANTISENSE OLGONUCLEOTIDE INHIBITS EXPRESSION OF ACETYLCHOLINE RECEPTORS. J.P. Albershek, II. Child Study Center, Yale University School of Medicine, New Haven, CT 06510.

In a preliminary effort to understand the transcriptional regulation of nicotinic acetylcholine receptor (nAChR) expression, a non-ionic antisense oligonucleotide was used to inhibit transport and/or translation of the mRNA coding for the four nAChR subunits.

Expression of nAChRs in muscle cells is regulated at the level of transcription of one of its four subunits, but is not well modeled in BChH1 cells, a myogenic line which expresses nAChRs when incubated in low serum media. To inhibit this expression, a 12-mer antisense oligonucleotide, an antisense oligonucleotide (Marcus-Sekura, C., Anal. Biochem. 172:289- 295, 1988) was designed using a "universal" nAChR DNA sequence as a guide (Buonanno et al., J. Biol. Chem. 251:16451- 16458, 1986). Mitotic BChH1 cultures were incubated in low serum in the presence of 0.4μM and 4.0μM oligonucleotide. Surface nAChRs were assayed at 2.5 days and 5 days using 125I-labeled bungarotoxin. The low oligo concentration had no effect compared to control, while the higher concentration reduced surface nAChRs by approximately 50%. Subunit-specific antisense oligonucleotides will provide a new tool for analysis of the regulation of nAChR transcripts.

353.5


To facilitate the study of the regulation of TH gene expression, a rapid, sensitive and quantitative method for measuring TH mRNA levels was developed. The assay is based on a ribonuclease protection of a 32P-labeled riboprobe (from a 380 bp cDNA of the rat TH mRNA sequence). After hybridization for 16 hrs at 75°C, resistant riboprobe is precipitated with TCA and filtered using a cell harvester. Analysis was by scintillation counting and quantitation of mRNA was by use of a standard curve. TH mRNA is present in each sample subjected to the TH mRNA assay. The hybridization conditions produced a linear curve from 2 to 125 pg of TH sense transcript to 10-100 pg of TH mRNA. Reserpine treatment (4 mg/kg sc, once daily for two days), produced in 24 hours after the second dose a 4-fold increase in adrenal TH mRNA (4.8 to 23.3 pg/ug RNA) in Lewis rats, a 4-fold increase in SD rats (5.3 to 21 pg/ug RNA) and an 8-fold increase in Syrian hamsters (14.5 to 36.8 pg/ug RNA). These results demonstrate the sensitivity and utility of this method for quantitation of TH mRNA. 

(Supported in part by NIDA Grants DA-01457 and DA-05130.)

353.6

APPEARANCE OF TYROSINE HYDROXYLASE-LIKE IMMUNOREACTIVITY AND TYROSINE HYDROXYLASE-LIKE mRNA IN CEREBELLAR PURKINJE CELLS OF THE MUTANT TOTTERING AND LEANER MOUSE. M.C. Austin, A. Abei*, J.T. trumpet*, I. Evarte, S.M. Paul, J.N. Crawley and M. Schultz. Clinical Neuroscience Branch, NIMH, Bethesda, MD, Dept. Vet. Biocience, University of Illinois, Urbana, IL. Tottering (tg) and leaner (gn) are two autosomal recessive mutations that are characterized by the development, at approximately 4 to 5 weeks of age, of spontaneous absence seizures, local motor seizures, and ataxia, which continue throughout adulthood. The tg allele has a severe focal motor seizures and ataxia compared to the gnt allele. Noebels (1986) reported that the major neurochemical abnormality in the brain of tg mouse is an adrenergic neuropathology in terminal regions of neurons located in the locus coeruleus (LC). Based upon this finding we examined tyrosine hydroxylase (TH) mRNA levels and TH-immunoreactivity in the tail of thoracic spinal cords from wild type and thg mice. Hybridization results did not reveal a significant difference in concentration of TH mRNA in LC neurons of thg and thg+ mice. However, we now confirm and extend the previous finding by Hess and Wilson (Nucleic Acids Res 39:1829-1849) of high levels of TH mRNA and TH immunoreactivity (IR) in the cerebellar Purkinje cells of these mice. We have found a significant increase in TH grain density and TH-IR in Purkinje cells of young (pre-seizure) and adult thg and thg+ mice. Also, Purkinje cells of young and adult thg and thg+ mice express TH mRNA and TH-IR, but at a much lower level. Northern blot analysis confirmed the findings from the in situ and immunohistochemical studies. Purkinje cells containing TH mRNA and TH-IR were more numerous in the caudal portions of the vermis and central portions of the folliculus and parafolliculus. These findings indicate that the expression of TH in cerebellar Purkinje cells of thg and thg+ mice is independent of seizure onset, and may be related to the genetic defect in this neurological syndrome.
535.7 MECHANISM OF INDUCTION OF mRNA FOR TYROSINE HYDROXYLASE BY MEMBRANE DEPOLARIZATION OF PC12 CELLS.
Membrane depolarization is a model of prolonged neuronal activity or stress. We studied the effect of 50 mM KCl or 150 mM veratridine, on the mRNA levels for
tyrosine hydroxylase (TH) and dopamine B-hydroxylase (DBH). TH mRNA increased up to five fold after continuous treatment, with 52 12 hrs with 50 mM KCl. Depolarization with 150 mM veratridine had a similar effect. In
contrast, DBH mRNA levels were unchanged by either KCl or veratridine treatment.
We conclude that either extracellular, or IP3 sensitive intracellular, pools of calcium may be required for the induction of TH mRNA in depolarized PC12 cells.

535.8 REGULATION OF TYROSINE HYDROXYLASE GENE TRANSCRIPTION IN PC18 CELLS BY CELL DENSITY. C. D.
Carlton and A. W. Tunks. Department of Pharmacology, University of
Rochester, Rochester, NY 14622.
Increasing cell-cell contact in rat pheochromocytoma PC18 cells elevates the levels of tyrosine hydroxylase (TH) and TH mRNA. In previous studies, we have shown that these increases are due at least partially to an elevation in the transcription rate of the TH gene. In this study, we further investigate the effect of cell density on TH gene transcription. When PC18 cells were cultured at high density (100 cells/cm2), the transcription rate of the TH gene is identical to that observed in cells cultured at low density (1 x 10° cells/cm2) for approximately 6-12 hours. After this prolonged lag period, the transcription rate of the TH gene in high density cells increases 2-3 fold over that observed in low density cells and remains elevated for at least 48 hours. This time course correlates well with that observed for the effect of high cell density on TH mRNA levels in PC18 cells. In addition, we have examined the role of cAMP in the density mediated induction of TH. The effects of high cell density and the cAMP analog, 8-chloro-34-thymidylinethionine, are additive on TH mRNA levels and TH gene transcription rate. These results suggest that in PC18 cells the cell-cell contact mediated stimulation of the TH gene is not elicited by a cAMP-dependent pathway. Furthermore, the time course of this effect on TH gene transcription shows a delayed onset; hence, this effect may depend upon the initial synthesis of factors that regulate the transcription rate of the TH gene.

Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27514.
Tyrosine hydroxylase (TH) is the rate limiting enzyme in the biosynthesis of dopamine, a transmitter in the body (CB). The present study was undertaken to ascertain the extent of gene expression for TH is increased in CB during hypoxia, a natural stimulus for CB. Adult rats were exposed to either hypoxia (10% O2 for 12 hrs) or hypothermia (control) for
5-10 hrs. Two groups of rats were used: normoxic and CB stimulated with CB. The CB was increased at all points in CB after hypoxia relative to control. In contrast, TH mRNA was not increased in response to hypoxia in the hypoxic group.

535.10 RAT TRYPTOPHAN HYDROXYLASE GENE EXPRESSION IN BRAINSTEM AND PINEAL GLAND. R. P. Hart, R. Yang* and L. A. Riley.
Dept. of Biol. Sci., Rutgers University, Newark, NJ 07102.
We have cloned a segment of the rat tryptophan hydroxylase gene using synthetic oligonucleotides from the CDA sequence (Durrant et al., J. Neurochem. 51: 312, 1988). The 5 kb region encodes five exons of the pineal CDA sequence. Exon sequences are completely homologous to the published CDA sequence, and the intron/exon junction positions match those predicted by homology with phenylalanine hydroxylase and tyrosine hydroxylase gene sequences.
Using oligonucleotides from the CDA sequence, we detect two species of mRNA on Northern hybridization (1.8 and 4.5 kb) in both pineal and brainstem RNA. The brainstem TPH mRNA is present at an equivalent low levels—detection was only possible with poly(A)+ RNA and long exposure times. However, TPH mRNA was easily detected in pineal total cellular RNA.
In order to begin to determine why such a discrepancy in TPH mRNA levels between pineal and brainstem, we utilized our cloned TPH gene sequences in a nuclear run-on assay for transcription. Results indicate similar levels of transcription from the TPH genes in pineal gland, medulla oblongata, and midbrain raphe regions. This indicates that levels of TPH mRNA are controlled post-transcriptionally. (Supported by NSF BNS 890551.)

535.11 STIMULATION OF PNMT mRNA EXPRESSION BY Ca2+ INFLUX. M. J. Evinger, T. H. Job and D. J. Reis.
We have demonstrated that specific neurotransmitters can alter steady state levels of messenger RNA for the epinephrine-synthesizing enzyme phenylethanolamine N-methyltransferase (PNMT). In primary bovine adrenal chromaffin cell culture, PNMT mRNA increases upon treatment with choline (nicotine and muscarine), histaminergic, and imidazole agonists, probably through activation of defined second messenger systems. Because certain neurotransmitter effects are mediated in part by increasing intracellular Ca2+ levels, we tested the hypothesis that elevated levels of calcium may independently stimulate production of PNMT mRNA. Incubation of bovine chromaffin cells with the Ca2+ channel blocker A23187 or the agonist BAY X 8644 (100 nM) elicited a 2-4 fold increase in PNMT mRNA detected by quantitative hybridization, increases consistent with and of greater magnitude than those produced by 20 mM Ca2+ in chromaffin cells, nicotinic receptors are positively associated with the influx of extracellular calcium. Likewise, blocking Ca2+ entry diminishes nicotine-induced increases in PNMT mRNA levels: the Ca2+ channel antagonists nifedipine, verapamil and thiadiazoxide as well as EGDME (10-4 to 10-5 M) degrees the PNMT mRNA response to 50 μM nicotine. Considering that Ca2+ also stimulates other adrenergic neuronal genes, notably sphenkephalin, these results collectively establish a regulatory role for calcium ion influx as an independent and significant neuromodulator of PNMT gene transcription.

Melatonin is a unique indoleamine synthesized in pineal and retina from serotonin. Circadian utilization of serotonin during darkness or adrenergic stimulation for melatonin synthesis is achieved through expression of the enzymes arylalkylamine N-acetyltransferase (NAT) and HIOMT. As a first step in the elucidation of the mechanism that regulates these enzymes and the genetic basis of circadian rhythms, we isolated CDA mRNA which encodes these pineal/retinal specific enzymes.
A rat pineal (RP) and retina (R) library (Craft et al., J. Neurochem., in press) were screened (1 x 10° plaques) with a cDNA probe for bovine pineal HIOMT (cBPH) (Isbida et al., 1987, JBC 262:2895). Plaque-purified recombinants (cBPH/1-6) were amplified by polymerase chain reaction (PCR) with +5'SK-KS closing site primers. Products were electrophoresed on a 1.5% agarose gel. Insert sizes were 0.4 to 2.1 kb. Sequence verification of cBPH/1-6 are being analyzed. PCR of the 2.1 kb cBPH paired with +/+BPH primers revealed bands of similar size to cBPH control. PCR of cDNAs synthesized from total rat pineal/retinal mRNA with +/+BPH primers revealed fragments of the predicted size in both tissues with lower levels in retina.
Society for Neuroscience Abstracts, Volume 16, 1990


We have studied the development of active membrane properties of a relatively pure population of cultured Purkinje cells, using whole cell patch clamp and fura-2 based (Ca^{2+}) microfluorimetry techniques, with a view to elucidate the role of Ca^{2+} in intracellular regulation in these neurons. Cerella from day 16 embryonic rats were dissociated and cultured in defined serum-free medium over a feeding layer of astrocytes. By immunocytochemistry using the mAb calbindin, which is specific for Purkinje cells within the cerebellum, approximately 90% of cells stained positively. Within the first several days in culture, these cells exhibited voltage activated Na+ and Ca^{2+} conductances and firing behavior. By the 17th day in culture the conductances were larger and the cells began to fire bursts of Na+ action potentials during current stimulii. During the second week in culture, trains of spontaneous action potentials became common, similar to the repetitive action potentials reported in Purkinje cells in slices (Kapoor, R. et al., Neurosci. 36:493-507, 1988). Sensitivity to glutamate, as indicated by a rise in [Ca^{2+}], was observed from the earliest times in culture and increased as the cells matured; these responses were gram at Mg^{2+}-free solutions. These cultured Purkinje neurons show an early progression towards mature cell characteristics.

536.4 CALCIUM SPIKES AND CALCIUM PLATEAUS EVOKED FROM DISTAL DENDRITES OF RAT SPINAL MOTONEURONES. J.R. Bronson*, C. Hounsgaard, and A. Chaffee. Dept. of Physiology, University of Copenhagen, 2100 Copenhagen, Denmark.

In motoneurones in transverse slices of the turtle spinal cord nitrendipine insensitivity Ca spikes are promoted by TEA while nitrendipine sensitive Ca plateaus are promoted by 5-HT and apamin (Hounsgaard et al. J. Physiol. 391: 575-589, 398: 591-603, 414: 265-282). We have used differential polarization by applied electric fields (Chan et al. J. Physiol. 402: 731-771 and 409: 145-156) to determine the compartmental origin of the two Ca mediated regenerative responses. During experiments the transmembrane potential was measured at the motoneurone soma while electric fields were established by passing currents between plate electrodes on either side of the preparation. Synaptic responses were minimized by the presence of TTX, APV, CNQX bicuculline, strychnine and picterotoxin.

In the presence of TEA electric fields in the ventrodorsal or the mediolateral direction could evoke Ca spikes independent of the polarity of the field and of the membrane polarization at the soma. In the presence of apamin Ca plateaux were generated by the same regime of differential polarization that was used to generate Ca spikes.

The results show that distal dendritic motoneurones can be shown to support Ca spikes and Ca plateaux. This suggests that voltage dependent current generators are involved in local processing of synaptic responses in the dendrites of motoneurones.

536.6 ATP RECEPTOR-OPERATED CA INFLUX AND 3H-NOREPINEPHRINE RELEASE IN PC12 CELLS. K. Inoue*, K. Nakazawa, and K. Fujimori, and A. Takahama. Div. Pharmacology, NIHs, 1-18-1 Kiyamiga, Setagaya, Tokyo 158, Japan

We have previously reported that ATP stimulates 3H-norepinephrine (NE) secretion in anion mechanism not coupled with voltage-gated Ca channels (Neurosci.Let.,106,294, 1989). We report here more details of this mechanism as determined using the bath method previously reported (J.Biol. Chem., 283, 8157, 1988), and the whole cell voltage-clamp technique. ATP stimulated intracellular Ca increase and NE secretion from PC12 cells in a dose-dependent manner which paralleled that of the ATP-activated current in the concentration range from 15 μM to 1 mM. The secretion and increase of intracellular Ca were dependent upon extracellular Ca, but were not inhibited by the Ca-channel blockers nicardipine (up to 10μM) or cadmium (up to 300μM). ATP-stimulated NE secretion was not influenced by an increase of extracellular Ca to 18 mM, but was inhibited by higher concentrations in accord with our report on the ATP-activated Ca current (J.Physiol. (Lond., in press, 1990). Secretion was inhibited by 10μM-guanethidine (30-300 μM). The rank order of potency of agonists in evoking the secretion was ATP > ATP-γ-S > ADP, β,γ-Methylyl ATP, AMP or adenosine had no effect. These findings suggest that extracellular ATP activates P2 receptors in PC12 cells and that the resultant Ca influx evokes NE secretion.


We have developed an immunoblotting technique to detect arginine vasopressin (AVP) in individual isolated nerve terminals from the neurohypophysis. After standard whole-cell patch-damp recording, the contents of terminals were sucked up into the recording electrode and stored at -20°C. The immunoblot control assays were prepared by blotting known amounts of three antigens (AVP, oxytocin and met-enkephalin) on the nitrocellulose membrane and then exposing to AVP-antibody. AVP showed reactions ranging from 50 pg - 10 ng, while oxytocin and met-enkephalin showed no reactions. A total of 59 terminals were assayed, 44 of which showed positive reactions and 15 of which showed no reactions. We were also able to characterize the Ca-currents of these individual isolated neurohypophysial terminals. This technique is uniquely designed to assist in direct identification of a variety of antigens and other markers from individual cells (or sub-cellular compartments such as terminals) after whole-cell patch-damp studies characterizing voltage-gated currents and drug effects, and should allow direct correlation between physiology and cell type. This research supported by PHS grant AA08003.

The presence of IA in certain pyloric neurons (PD, AB, PY) suggests that this conductance could influence cycle frequency and bursting of central pattern-generating systems. We used 4-aminopyridine (4-AP; 1 mM) to reduce IA in cells within the intact circuit and in individual putative pyloric neurons isolated from other pyloric neurons. In the intact circuit, 4-AP always increased cycle frequency ($X = 27%$, $n = 8$). 4-AP also altered phase relationships of neurons relative to the PD burst onset. In the isolated cell, spikes/burst and frequency within bursts were significantly increased by 4-AP. Changes in oscillation amplitude were consistent, except in the AB neuron where oscillation amplitude was increased during 4-AP application (4.5 mV; $n = 4$). When cells were isolated from other pyloric neurons (using photoactivation and PTX; no naclo block) 4-AP enhanced or reduced burst activity in all cells. These data are consistent with the hypothesis that cells thought to lack significant IA (LP, VD, IC) may possess this conductance in nonstomacic cell regions. Supported by NIEHS #NS05837-01 (AJT) and NIH #NS17323 (RMH-W).

CALCIUM DEPENDENCE OF SPIKE REPOLARIZATION IN RAT MAGNOCERULAR NEUROSECRETORY CELLS (MNCS). K. Kirkpatrick and C.W. Bourque. Centre for Research in Neuroscience, Montreal General Hospital and McGill University, Montreal, Canada H3C 1A4.

Activity dependent changes in spike duration in MNCS result from the variable expression of a Ca++-component (Bourque and Renaud, 1985, J. Physiol. 363). This study reveals an additional role for Ca+ in controlling spike duration. Intracellular recordings were made from 37 supraoptic nucleus MNCS in hypothalamic explants superfused with artificial CSF (32°C). Single spikes were elicited at 0.5 Hz from a fixed subthreshold membrane potential by applying 8 ms depolarizing pulses. Addition of Ni2+ or Cd2+ (100-400 µM, n=17) or removal of extracellular Ca2+ (n=8) consistently and reversibly decreased spike duration. In contrast, raising [Ca2+] from 2 to 4 mM had either no effect or increased spike duration (n=4), but increased the rate of spike repolarization. This apparent paradox suggests that Ca2+ influx may contribute to the activation of repolarizing currents. In agreement, intracellular injection of BAPTA, a Ca++ chelator, progressively increased spike duration (n=8) in concert with the disappearance of the AHP. These results suggest that a rapid Ca2+-dependent K+ current may contribute to spike repolarization in MNCS. Supported by FRSQ and MCB.

Ia AND RHYTHMIC FIRING IN IDENTIFIED VISUAL CORTICAL NEURONS. J. Solomon and J.M. Nerbonne. Dept. of Pharmacology, Washington University Medical School, St. Louis, MO 63110.

We have previously demonstrated that α-adrenergic agonists attenuate a hyperpolarization-activated inward current, IA, in layer V superior colliculus-projecting (SCP) neurons of rat primary visual cortex. Because norepinephrine disrupts rhythmic firing of lower layer cortical neurons in vitro, we are investigating the role of IA in patterning the response of SCP cells to inhibitory inputs. Dissociated SCP neurons from postnatal day 7-13 Long-Evans rats were identified in vitro following in vivo retrograde labeling with rhodamine beads. Whole-cell recordings were obtained within 48 hours after isolation. 10% SCP cells, hyperpolarizations from a holding potential of -40 mV evoke an instantaneous, noninactivating inward current; steps negative to -70 mV also reveal the slowly activating IA. The current is inward when potentials more negative than -90 mV (n=5) and IA is selectively and reversibly blocked by 3 mM extracellular Cs++ (n=7). The rates of rise of IA are best fit by single exponentials with mean (±SEM) activation time constants of 1.88±0.74 and 0.45±0.22 sec at -80 mV (n=7) and -110 mV (n=11), respectively. Under current clamp, the activation of IA attenuates nonlinear repolarization for IA. Under voltage clamp, the deactivation of IA on removal of the hyperpolarizing current augments the firing of action potentials. IA, therefore, appears to be important in the efficacy of the inhibitory inputs on SCP neurons and patterning cell firing following release from inhibition. (Supported by NSF #BNS 880923 and NIH #5T32 GM07805).


The divalent cation Ni2+ has been shown to specifically block T-type calcium channels in mammalian neurons. However, the effects of this ion on invertebrate neurons are relatively uncharacterized. The objective of this study was to determine the effects of Ni2+ on pyloric neurons of the spiny lobster, Panulirus interruptus.

The stomatogastric ganglion was maintained in vitro at 14°C and perfused with chloride saline. Data were obtained from post synaptic somata using 2 electrode current clamps. TTX was added to the saline to eliminate spikes and rhythmic activity.

Concentrations of 50-100 µM Ni2+ induced voltage oscillations of similar amplitude and frequency to the endogenous rhythmic activity. Lower concentrations of Ni2+ (25-50 µM) increased the input resistance of the neurons, enhanced both synaptic and electrical transmission and altered the voltage waveforms. These effects are qualitatively different from the simple blockade of an InM Ca2+.

These results indicate that Ni2+ exerts qualitatively different effects on crustacean and mammalian neurons. Supported by NS15697.

MODULATION OF MOTONEURON REPETITIVE FIRING BY MULTIPLE OUTWARD CURRENTS. F. Viana and A.J. Berger. Dept. of Physiology & Biophysics, Univ. of Washington School of Medicine, Seattle, WA 98195.

We examined the firing properties of hypoglosal motoneurons in tranverse brainstem slices (50 µm) from neonatal mice. Depolarizing current pulses from rest (-60 to -70 mV) evoked repetitive firing that showed a decrementing pattern (adaptation). Hyperpolarizing voltage-clamp pre-pulses (1 sec) caused an early decrease in firing rate, which then reversed to an accelerating type of discharge pattern. At slow firing frequencies (2-4 Hz) the hyperpolarizing pre-pulse caused a significant delay to the first spike (delayed excitation). Solutions with nominally zero Ca2+ (0.5 mM EGTA) or addition of 300 µM Cd2+ caused an approximate two-fold increase in the slope of the steady-frequency-current relationship but did not prevent the early decrease in firing and the delayed excitation caused by the hyperpolarizing pre-pulse. The spike duration, including the fast repolarization, was virtually unaltered by removal of Ca2+, but the medium duration afterhyperpolarization was abolished.

In voltage-clamp, after TTX, removal of Ca2+ caused a strong reduction of the steady-state outward current evoked by depolarizing voltage steps. Hyperpolarizing pre-pulses resulted in removal of inactivation of a slow transient outward current not blocked in Ca2+-free solution. Both outward currents decreased in amplitude with high extracellular K+. In some cells, 4-AP (500 µM) caused only modest reduction of the transient current. We conclude that the pattern of discharge of hypoglosal motoneurons is modulated by voltage- and Ca2+-activated potassium currents. These results may prove relevant for aspects of synaptic integration and recruitment timing. (Supported by NS 14857).

DIFFERENT ELECTROPHYSIOLOGICAL CELL TYPES IN ENTRAPED CORTEX LAYER II IN RAT BRAIN SLICES. A. Alonso and R. Bilbao. Dept. of Neurology & Neurosurgery, McGill University, Montreal, Canada, H3A 2B4 and Dept. of Physiology & Biophysics, NYU School of Medicine, New York, N.Y. 10016.

Intracellular recordings were obtained from medial entorhinal cortex (MEC) neurons. Two distinct cell types were identified on electrophysiological and morphological bases. The more abundant (65%) cell type (type 2) corresponded to the "large entorhinal cells" characteristic of “dyschogenic” layer II (Alonso & Milner, Nature 342: 175-177, 1990). These neurons displayed a pronounced time-dependent anomalous rectification due to a Q-like current) and rhythmic subthreshold independent membrane potential (9%). Oscillations (7-12 Hz) which persisted the blockage of Ca++ conductance. The second cell type (type 1) also had a cell-like morphology but with one primary dendrite being thicker and longer than the others. Type 2 differentiated from type 1 cells by their slower action potentials, larger afterhyperpolarizations, weaker non-hyperpolarized delayed firing upon depolarization. They also displayed subthreshold oscillations which were insensitive to low and of lower frequency than those in type 1 cells and which demonstrated a dual independent membrane potential (9%). Type 2 cells were more abundant than type 1 and the 2 types neurons could both be distinguished by their sensitivity to cholinergic agonists which induced dynamic changes only on type 2 cells membrane properties. In these neurons, the presence of carbachol (40µM) induced a hyperpolarizing afterhyperpolarization. Substrates for low-frequency plateau depolarizations that sustained repetitive firing. Moreover, weak constant current injection induced slow rhythmic depolarizing activity. Because of the key anatomical position of EC layer II, these two cellular types will significantly contribute to the network behavior of the hippocampal system and may underlie the generation of the two known distinct types of limbic theta rhythms.

536.9

536.10

536.11

536.12
ION CHANNELS: CELL FUNCTION

136.13

EFFECTS OF NEOCYCIN AND PHENYCYCLIDINE ON TWITCH TENSION
AND NERVE TERMINAL CURRENT AFTER POST-TETANIC POTENTIAI-
ATION. H.-C. Tsai and W.L. Chen*. Pharmacological Institute,
College of Medicine, National Taiwan University, Taipei, Taiwan.

Post-tetanic potentiation (PTP) is a transiently increased
sensitivity of the motor nerve and the increase in amount of
transmitter release is responsible for the PTP. In the present study, the effects of
neocycin and phenycyclidine on the twitch tension and
nerve terminal current after tetanic stimulation were
studied on the isolated phrenic nerve diaphragm and m.
trapezius muscles of mice. Neocycin and phenycyclidine
were associated with less post-tetanic potentiation of
twitch tension if the duration of tetanic stimulation was
less or 20 sec. Both compounds affected neither the
compound action potential of phrenic nerve nor the sodium
and potassium currents of the nerve terminal after
repetitive stimulation. However, neocycin decreased while
phenycyclidine does not decrease the calcium current in
the nerve terminal after repetitive stimulation. The
results suggested that neocycin affected the PTP by
tetanic tension by pre-synaptic mechanism while
phenycyclidine affected the PTP by post-synaptic
mechanism. (Supported by National Science Council, R.O.C.)

136.15

WHOLE-CELL PATCH CLAMP ANALYSIS OF THE PASSIVE
MEMBRANE PROPERTIES OF HIPPOCAMPAL PYRAMIDAL
NEURONS. H. Banerjee and D. Johnston, Division of Neuroscience,
Baylor College of Medicine, Houston, TX 77030.

Passive membrane properties are important determinants of
electrical responses in neurons with extensive dendritic arborizations. Intracellular micro-
detect recordings have revealed that the specific membrane resistance (Rm) of
hippocampal neurons is relatively high. However, the whole cell clamp technique
introduces an additional component to the leak conductance that may affect the passive
membrane properties of the cell. We have used patch clamp recording of
hippocampal neurons to test the hypothesis that this small leak conductance results
in an underestimation of Rm.

Hippocampal neurons were acutely isolated as described by Gray and
John-
son (Nature 327:490-492, 1987). Whole cell patch clamp recordings from dentate granule neurons
revealed a rapid decrease of input resistance (Rm) and membrane time constant (τm)
during the course of the experiment. Since the measured values of Rm and τm are directly dependent on Rm, it is likely
that the observed rundown is mediated by a decrease in Rm. This rundown could be prevented by including an ATP regenerating system in the pipette or by using perforated-patch recording, suggesting that a conductance mediated by ATP-
dependent K+ channels may develop during cytoplasmic dialysis.

Under conditions where stable recordings of passive responses were obtained,
we measured a τm of 4027 ms (mean±SEM; n = 6) for dentate granule neurons and 3546 ms (mean±SEM; n = 6) for CA1 pyramidal neurons. These find-
ings suggest that Rm may vary significantly from one neuron to another.

(Supported by grants MH44744, NS11535, and ASFR8-0142.)

136.16

MATHEMATICAL MODELING OF THE SEROTONINERGIC MODULATION OF
ELECTROPHYSIOLOGICAL PROPERTIES OF HIPPOCAMPAL
NEURONS. A. Kay, D.A. Baxter, and J.H. Byrne, Department of Neurobiology
and Anatomy, Univ. of Texas Medical School, Houston, TX 77225.

Serotonin (5-HT) enhances excitability and broadens the spatial
distribution of neuronal responses in the somata of pleural sensory neurons. In order to assess
the relative contributions of the serotonergic modulation of three K+ currents
make to the overall effects of 5-HT, we have constructed
the Hodgkin-Huxley type membrane model. The model consists of a passive
capacitance, a leakage conductance, differential equations that describe
eight membrane currents, and a description of intracellular Ca2+.

The parameters were adjusted to simulate the characteristics of
the membrane currents, spike, and excitability that are observed normally.

The 5-HT-dependent actions of 5-HT were simulated by reducing
the maximum conductance of the S K+ current (gKs) and a slow compo-
nent of the Ca2+-activated K+ current (gK1), whereas the CaMP-
dependent action of 5-HT was simulated by slowing the kinetics of the
delayed or voltage-dependent K+ current (Iv) (Baxter & Byrne 1989). The simulated effects of 5-HT were more than doubled excitability
but produced only modest spike broadening. The subsequent modula-
tion of gKs doubled the duration of the simulated spike, without fur-
ther increasing excitability beyond that produced by the
CaMP-dependent action of 5-HT. This results are remarkably
similar to the physiological actions of 5-HT and 5-HT,
and support the hypothesis that the CaMP-dependent actions of 5-HT play a key
role in regulating excitability, whereas the CaMP-independent action of 5-HT has an important role in regulating the duration of the spike.

Supported by Grant AFSOR 87-0274.

136.17

A MODEL OF THE CA3 HIPPOCAMPAL PYRAMIDAL CELL BASED ON
Watsco Res. Ctr., Yearnhtoks Heights, NY 10595, Dept. of Neurology,
Columbia University CPS, NY, NY 10032, and Institut Pansar, Paris, France.

It is desirable to have accurate models of CNS cells to understand the role
of active membrane currents in synaptic integration and plasticity, and in deter-
mining different firing patterns, and to use as building blocks for network models.

We have constructed a model of the CA3 cell using a uniform cable, with 6
spatially distributed active conductances: gL (4), gL, (5), gL, (3), gL, (2),
gL, (1), and gL, (3). The last 2 currents are Ca dependent; C-current is also
voltage-dependent. The conductances were derived from a population of pyramidal cells;
the kinetic model was constructed from the responses of CA3 pyramidal cells. We
used the Hodgkin-Huxley equations to model the amplitude and
phases of the recorded action potentials. The Hodgkin-Huxley equations and
many models of bursting neurons. To zeroth order, one
should consider the influence of the
results obtained from these models
on the realistic extent possible
the Hodgkin-Huxley equations and
the membrane potential and conserving stability at the
near the resting potential.

When applied to models of neurons containing many conductances, this method provides
direct insight into the mechanisms underlying burst
generation in pacemaker cells. This method is computationally
when such models are used to construct networks.

Supported by MH 46742 and T32 NS07292.
Large-Scale Compartment Model of a Cerebellar Purkinje Cell, P.C. Bush and T.J. Szewkowski. Salk Institute, La Jolla, CA 92037.

Cerebellar Purkinje cells have a variety of active conductances on both the soma and dendrites. These conductances produce a characteristic firing pattern in response to a depolarizing current pulse injected by a microelectrode at the soma. A computer model of a Purkinje cell has been constructed consisting of over 1000 compartments (Shelton 1993) and seven different conductance types. Instead of using Hodgkin-Huxley kinetics, a multivariable system difficult to apply in the absence of exact voltage and current traces, we developed a channel model based on Markov chain kinetics, a system with fewer variable parameters that is easy to understand and manipulate.

The model reproduced the firing pattern of real cells in response to current input as reported by Llinás et al. (1980): A slow depolarization due to sodium and calcium plateau currents causes an accelerating train of sodium-dependent action potentials at the soma. A high-threshold calcium-dependent spike is triggered in the dendrites just at the point of inactivation of the sodium spike train. Voltage- and calcium-dependent potassium currents then produce a large hyperpolarization which reactivates the sodium spikes, allowing the cycle to begin again. This cyclical firing pattern has a period on the order of hundreds of milliseconds, whereas single synaptic potentials last only for tens of milliseconds. Trains of EPSPs are being modelled to establish roles for these conductances under physiological conditions.

5HT3 RECEPTORS

5HT3 RECEPTORS


Seroferonic 5HT3 receptor blockade antagonizes the secretion of dopamine in rat brain suggesting a potential antipsychotic property of 5HT3 antagonists. [3H]quipazine labels 5HT3 receptors in rat cortex (Milburn C.M. et al., J. Neurochem., 52:1787, 1989). Displacement of [3H]quipazine binding by the selective 5HT3 antagonist ICS 205-930 in human amygdala showed a high affinity site of 0.25±0.03 nM and a low affinity site of 662±130 nM. We performed saturation studies with [3H]quipazine (0.25 to 20 nM), using ICS 205-930 (100 nM) to define the non specific binding. In the frontal cortex and caudate, no specific binding was detectable. In amygdala, the Ligand analysis of the Scatchard plot was compatible with a one site model (n=2): Kd= 5.8±0.5 nM, Bmax=42.6±5.8 fmol/mg of Protein. Similar results were obtained in human hippocampus. The labelled site in human amygdala and hippocampus is comparable to [3H]quipazine binding site to 5HT3 receptor in rat brain. This confirms the selective localization of these sites in human brain as previously described with [3H]zacopride (Baines, J.M., J. Neurochem., 52:1787, 1989) and suggests a rationale for the investigation of the antipsychotic properties of 5HT3 antagonists.

537.3

BLOCK OF CISPLATIN-INDUCED EMESIS BY SEROTONERGIC (5HT3) ANTAGONISTS IN SUNCUS MURINUS. N.Matsuki, Y.Tori, M.Muto and H.Saito. Dept. of Chem. Pharmaco., Fac. of Pharm. Sci., Univ. of Tokyo, Tokyo 113, Japan.

We have shown previously that the Suncus murinus (house mouse shrew), a species of insectivora, vomits in response to various emetic stimuli including cancer chemotherapy agents. In the present study emetic responses induced by cisplatin were characterized and effects of anti-serotonergic drugs were studied. Intravenous or intraperitoneal injection of cisplatin elicited vomiting responses dose-dependently. The ED50 values for intravenous and intraperitoneal administration of cisplatin were 8.4 and 10.0 mg/kg, respectively. Vagotomy completely abolished cisplatin-induced emesis. Di-iso-opi complex (DIA), which is considered as an active form of cisplatin, similarly induced emesis with significantly shorter latency.

Selective serotoninergic 5HT3 antagonists (ICS205-930, zacopride, BRL43994, GR38032F) strongly prevented cisplatin-induced emesis whereas the drugs were ineffective against veratrine, copper sulphate and motion stimulus. Intravenous or intraperitoneal injection of serotonin causing vomiting was prevented by ICS205-930 and zacopride. These results suggest that cisplatin is converted to DIA and releases peripheral serotonin which subsequently induces emesis through peripheral 5HT3 receptors.

537.4


We have previously described the action of bufotenin, at the 5-HT3 subclass of serotonin receptors. In functional assay systems bufotenin acts as a partial agonist (Cramer et al., IUPHAR, 1990). In this study we have pharmacologically profiled 2-methyl-bufotenin (2-Me-BUFO).

2-Me-BUFO showed marginal selectivity for 5-HT3 receptors, over 5-HT1A, 5-HT2A, 5-HT1D and 5-HT1D receptors in conventional ligand binding assays. Ks values were, respectively 0.036±0.030, 535, 625, 4360 mM. 5-HT has previously been shown to depolarize vagal nerve fibres in vitro at an action at 5-HT3 receptors (Ireland, S.J. and Tyers, M.B., Brit. Pharmacol., 90:229, 1987). However when assayed as a depolarizing agent, 2-Me-BUFO was only a weak partial agonist in comparison to 5-HT. The depolarizing effect of 2-Me-BUFO was sensitive to antagonism by ondansetron (GR 38032F); log Ks = 8.92 ± 0.07 (4). Further 2-Me-BUFO (1 μM) was capable of antagonizing the depolarizing response to 5-HT: log Ks 6.10 ± 0.06 (4).

In conclusion although 2-Me-BUFO shows some specificity for 5-HT3 receptors, it is unlikely to be a useful 5-HT3 receptor specific agonist.
SOFI 5HTR RECEPTORS

5HT 5-HT6350, 5H-BRL4364, and 5H-QUIPAZINE BINDING TO BOVINE AREA POSTREMA 5HT, RECEPTORS: AN IMPROVED RADIOIOUGAND BINDING ASSAY. M. Teller and S. Le Page, Dept. of Pharmacology and Toxicology, Albany Medical College, Albany, NY, 12208

Brain 5HT receptors have been detected using radioligand binding methodology (see ref. 1). However, the specific signal produced has either a) retained too much of the cell's ability to allow routine iontophoresis of drugs or detailed examination of the molecular characteristics of 5HT receptors. b) required a radioligand not commercially available, or c) required the development of a new and more practicable. A recent autoradiographical study revealed a high density of 5HT receptors in human area postrema (1). Therefore we decided to investigate the possibility of developing a reliable 5HT receptor radioligand binding assay in bovine area postrema homogenates.

Affinity (nM) Bond (% Specific) 5-HTR 6350 0.7 93 5H-BRL 4364 2.1 77 5H-QUIPAZINE 0.5 66

The preliminary data presented in the table was derived using 3 mg wet weight bovine area postrema homogenates and 10,000 cpm 5HT as the non-specific determinant. The high levels of binding and the high % specific binding obtained using 5H-BRL and 5H-QUIPAZINE suggests that the assay using these radioligands could be readily adaptable; the use of bovine tissue and commercially available radioligands (New England Nuclear) greatly increase the convenience of using the 5HT receptor on a routine basis. A detailed characterization of this binding system is now underway.


5HT-Binding Responses to Serotonin in the CA1 Region of the Hippocampus. Y. Chaput and R. Andrade. Dept. of Pharmacol, St. Louis Univ. School of Medicine, St. Louis, MO 63104

We have previously shown that 5HT, acting on receptors that do not belong to the 5-HT1, 5-HT2, or 5-HT3 subtypes, elicits a marked reduction of calcium-activated hyperpolarization (AHP) present in CA1 hippocampal neurons. We have now used intracellular recording techniques in slices to further characterize the pharmacology of this response.

Bath administered 5-HT dose-dependently reduced the AHP in the range of 10-100 nM with an EC50 of 3-10kM while methyloxyphenyl-2 methyl-5HT and methyloxyphenyl-3-carboxamidotryptamine were less potent than 5HT. In contrast, the 5-HT1a receptor agonist 2-methyl-5HT (300nM) and phenylisobutyline (300nM) were without activity at this receptor. Administration of the gastrokinetic benzenes 24924 (1,000nM), clonidine (100nM) and apomorphine (300nM) blocked the effect of 5HT on the AHP without altering the AHP themselves, while the neuroleptic benzamide sulphide was without effect.

These results support previous studies suggesting that the receptor mediating the decrease in the AHP in the hippocampus does not belong to be 5-HT1, 5-HT2, or 5-HT3 subtypes and demonstrates the existence of 5-HT-like receptors in the adult rat CNS capable of mediating slow excitatory responses to 5HT. Supported by Grant MH49860, an MRC postdoctoral Fellowship to Y.C. and the Sloan Foundation.

5HT6 5-HTR ANTAGONISTS FAIL TO INFLUENCE THE INTRAVENOUS SELF-ADMINISTRATION OF COCAINE OR AMPHETAMINE BY LABORATORY RATS. R. Peltier and S. Schenk, Texas A&M Univ., Dept. Psychol., College Station, TX 77843

It has been suggested that 5HT-receptor antagonists may be useful in the treatment of drug abuse. To assess this possibility, we compared the effects of two antagonists, IC550=9.0.01, 0.1, 1.0, mg/kg, IP) and 1938032F (0.1, 0.1, 1.0, mg/kg, IP), with the specific D2 dopamine receptor blocker, haloperidol (0.125 mg/kg, IP), on the intravenous self-administration of cocaine (0.5 mg/kg/infusion) or amphetamine (0.05 mg/kg/infusion). Neither of the serotonin antagonists altered self-administration. In contrast, haloperidol increased reinforced responding, suggesting a shift to the right in the dose/response curve. These data fall to support a role for the serotonin 5HT3 receptor system in the reinforcing properties of psychostimulants.

5HT7 5-HT6 INDOLETHYLAMINE INDUCED DA RELEASE IN THE NUCLEUS ACCUMBENS MEDIATED BY THE 5HT6, RECEPTORS. L.H. JIANG, L. FISHKIN, S.X. TIAN AND R.Y. WANG. Department of Psychiatry and Behavioral Sciences, SUNY at Stony Brook, Stony Brook, NY 11794-8790

Using the technique of chronocoulometric recording, we have previously shown that the intravenricular (icv) administration of the 5HT_6 receptor agonist 2-methyl-5HT (2-Me-5HT) dose-dependently increases the release of DA in the nucleus accumbens (NAc). The present study was to characterize further the effect produced by 2-Me-5HT. Sprague-Dawley rats were anesthetized with chloral hydrate. A Nafion coated carbon-fiber electrode was calibrated in vitro and then was implanted in the NAc. The ratio for DA/AA and DA/DOPAC obtained from Nafion coated carbon-fiber electrodes in the NAc of Sprague-Dawley rats was 98±7% and 57±4, respectively, indicating the electrodes were highly selective for detecting DA. Moreover, addition of 5-HT or 2-Me-5HT into the buffer solution did not produce the signal, indicating the DA but not 5-HT is being detected. A computer-aided in vivo electrochemical system (Cypres system) was used to monitor the release of DA. As reported previously, icv injection of 2-Me-5HT dose-dependently increased DA release in the NAc. In contrast, icv administration of the antagonist saline (n=5), 5-HT_6 (n=5) or 2-Me-5HT antagonist DOI (n=3) was without effect. 2-Me-5HT-induced increase of DA release could be blocked by low injection of 5HT_6 receptor antagonist (n=9) and IC50=4. However, icv administration of 5HT_6/5HT_3 receptor antagonist metipipramine failed to significantly block the electrochemical signal elicited by 2-Me-5HT. These results strongly suggest that the 2-Me-5HT-induced DA release in the NAc is mediated by 5HT_6 receptors in the rat brain. (Supported by USPHS Grants MH-41440 and MH-00378).

5HT8 EFFECTS OF THE 5-HT6 RECEPTOR ANTAGONIST 2-METHYL-5HT ON THE NEURAL ACTIVITY OF A10 DA CELLS. R.Y. WANG AND L.H. JIANG. Department of Psychiatry and Behavioral Sciences, SUNY at Stony Brook, Stony Brook, NY (1974-8790)

We have previously reported that the intravenricular administration of the selective 5HT_6 receptor antagonist 2-methyl-5HT (2-Me-5HT) dose-dependently increased the DA release in the nucleus accumbens (NAc). Moreover, 2-Me-5HT-induced effect depended upon the impulse flow of DA neurons. The aim of present study was to determine whether the effects of 2-Me-5HT on the DA release can be explained by its action on the firing rate of A10 DA cells. The techniques of single cell recording and microiontophoresis of DA cells in chloral hydrate anesthetized Sprague-Dawley rats. The concentration of the drugs in the multiwell electrodes was 10 mM. Of the 8 unidentified A10 DA cells tested with 2-Me-5HT, 33 (37.5%) were excited, 52 (59%) were not affected and 3 were inhibited. Of 12 antidromically-activated 10-NAc DA cells, 10 (83%) were activated by 2-Me-5HT. The rest were not affected. Iontophoresis of 2-Me-5HT onto non-DA cells (n=10) in the ventral tegmental area was without effect. Both 5HT6-receptor antagonists (ip=10 and NCS=3) but not 5HT_3/5HT_6 antagonists ritanserin or metergoline blocked the 2-Me-5HT excitatory action (p<0.05, t test). In addition, prolonged iontophoresis of high concentration of magnesium, which presumably blocked the 5HT_6 receptors, did not alter the excitatory action of 2-Me-5HT (n=8), suggesting the effect is a direct one. Our results indicate that 2-Me-5HT-induced increase of DA release in the NAc could be activated by 5HT6 receptor action on the A10-NAc DA neurons. (Supported by USPHS Grants MH-41440, MH-00378).
537.11

We have reported that the iontophoretic application of the 5-HT3 agonist 2-methyl-5H-imidazoline (2-Me-SHT) suppresses the firing of medial prefrontal cortical (mPFC) cells and this effect is blocked by the selective 5-HT3 antagonists granisetron and ICS 20930 (Ashby et al., Eur. J. Pharmacol., 173, 196, 1989). We here report the further characterization of the 5-HT3 receptors in sham-operated Sprague-Dawley rats, using the techniques of single unit recording and microiontophoresis. The microiontophoresis of 2-Me-SHT and phenylbiguanide (PBG) produced a current-dependent (10-80 nA) suppression of spontaneously active mPFC cells firing, with the effect of 2-Me-SHT being greater than that of PBG. The iontophoresis of 2-Me-SHT (0.5 - 20 nA) also produced a current-dependent suppression of 1-glutamate-activated mPFC cells. The microiontophoresis of 1M MgCl2 for 15-20 mins. did not alter 2-Me-SHT's suppressive effect, suggesting that its action is direct. The suppressant action of 2-Me-SHT was blocked by the selective 5-HT3 antagonists granisetron, ICS 20930, LY 278384, MDL 72222, ondansetron and (1-zacopro) at currents of 5-10 nA but not by the antagonists 1-sulphide (D) and (2) propanolol (5-HT-A), indani (5-HT1c), H, metoprolol (3HT1, 2, 3, 4, SCH 23390 (D, 5HT1c, 2 and SR 5910 (GABA) +). The rank order of effectiveness for the 5-HT3 antagonists to block 2-Me-SHT action is: ICS 20930 > zacopro > granisetron > LY 278384 > ondansetron > MDL 72222. Overall, our results indicate that 2-Me-SHT's suppressant action is directly mediated by 5-HT3 receptors. (Supported by USPHS grants MH-41440, MH-00378 to R.Y.W. and MH-0951) to C.R.A.)

537.13

The 5-HT3 receptor is presumed to mediate the excitatory actions of serotonin in the peripheral nervous system. Initially, it was believed that 5-HT3 receptors were present only in the periphery, but an increasing body of evidence indicates their presence in the brain. In order to localize 5-HT3 receptors in the rat brain, we have utilized the recently introduced high affinity antagonist ligand, [3H]-LY278544.

Twelve micron tissue sections of rat brain were utilized for a variety of biochemical studies to determine the appropriate conditions for labelling to slide mounted tissue section. Nonspecific binding was determined by the addition of 10 μM 5HT in the incubation buffer. Following these experiments, labeled sections were exposed to autoradiographic film for 2-6 months.

[3H]-LY278544 bound to tissue sections with a Kd of 0.69 nM and a Bmax of 79 fmol/mg tissue and was displaced by 5HT and 5HT3 specific antagonists. The autoradiographic detection of [3H]-LY278544 binding indicated a widespread distribution of the 5-HT3 receptor in the brain. The highest receptor densities were detected in the area postrema and nucleus of the solitary tract followed by high levels in the substantia gelatinosa of the trigeminal nucleus and spinal cord. Moderate levels of binding were found in the dorsal motor nucleus of the vagus, parafincomum cortex, olfactory bulb, superficial laminae of the cerebral cortex and the caudal hippocampus. These results indicate that [3H]-LY278544 is a useful tool to study 5-HT3 receptors in the brain by autoradiography.

537.14
EFFECT OF 5HT, AGONISTS ON IN VITRO [3H]-5HT EFFUX. G. M. Williams, L. D. Smith*, and D. J. Smith, Deps. of PCL & Toxicol. and Anets., WVU, Morgantown, WV 26506. 5HT receptors appear to be present on rat spinal synaptosomes (ETP 156-287, 1988). and pre-synaptic 5HT receptors have been shown to modulate neurotransmitter release (H. N.L and R. Y., 9:199, 1986). The current study asks if release-modulating 5HT receptors are present on spinal 5HT nerve terminals. A synaptosomal fraction was isolated from rat spinal cord, incubated with 100 nM [3H]-5HT, and aliquots superfused at 37°C with TRIS-buffered Krebs solution. A pre-drug fraction and a duration drug fraction were collected from each superfusion chamber. 5CH,5HT and phenylbiguanide (PBG) did not alter basal efflux at concentrations < 1 μM. At concentrations > 100 μM butrofenone increased basal efflux of both 5HT and 5CH. 5HT and haloperidol, produced a supression of basal efflux. The effects of Na+ were also observed in hippocampal and striatal slices. In contrast the responses to carbachol and ibotenate were similar in medium containing 2 or 5 mM Na+. These results demonstrate the importance of Na+ concentration may differentially influence the effect of agonists on phosphate-induced hydrolysis.

SECOND MESSENGERS VI

558.1
INDEPENDENT EFFECTS OF pH, Ca2+ AND PHOSPHODIESTERASE ON THE cAMP-GATED Na+ CURRENT IN NEURONS OF THE MOLLUSCAN PLEUROBRANCHAENAE. V. Mazzarella and R. Gillette, Neuroscience Program and Dept. of Physiol. & Biophys., Universtity of Illinois, Urbana, IL 61801. Na+ current gated by cAMP (IcAMP) is the ventral cell body cicular neuron of Pleurobranchaea is sensitive to changes in intracellular pH (pHi), extracellular Ca2+, and phosphodiesterase activity (PDE). cAMP can increase IcAMP through a variety of intracellular events. From experiments measuring the IcAMP response to injected cAMP, we find that a number of factors that can influence pH or Ca2+ can affect IcAMP. We find increases in pH decrease IcAMP, whereas increases in Ca2+ increase IcAMP. We have also found that addition of activators of PDE's decreases IcAMP. We also find that increases in Ca2+ increase the activation of PDE and will increase the sensitivity of IcAMP to changes in Ca2+. These results suggest that the activity of the PDE may be a direct function of the Ca2+ influx into the cell. The effects of Ca2+ on IcAMP are independent of the effects of pH on IcAMP. The results of these experiments suggest that a mechanism other than a pH effect on PDE causes the increase in current amplitude. We have also investigated the role of extracellular Ca2+ on IcAMP. We find that extracellular Ca2+ has a direct effect on IcAMP. This effect is independent of any changes in IcAMP caused by changes in pH or Ca2+. We also find that the increase in amplitude caused by activation of an extracellular factor is independent of the activation of PDE. Removal of extracellular calcium increased IcAMP amplitude but not the decay rate. When pH was decreased under these conditions, the increase in amplitude was observed, indicating different modulation mechanisms for calcium and pH.

558.2

The hydrolysis of inositol-containing phospholipids is an important signal transduction mechanism in the brain. Phosphoinositide hydrolysis in vitro is known to be influenced by the concentration of both K+ and Ca2+ ions. In this study using rat brain slices we measured the effect of altered Na+ concentrations on phosphoinositide hydrolysis induced in response to a number of agonists. Reductions of the Na+ concentrations below 120 mM resulted in increased increases in basal and norepinephrine stimulated accumulation of [3H]-inositol monophosphate in cortical slices that had been prelabelled with [3H]-inositol, and maximal responses were obtained with 0 and 5 mM Na+. The effects of Na+ were also observed in hippocampal and striatal slices. In contrast the responses to carbachol and ibotenate were similar in medium containing 120 or 5 mM Na+. These results demonstrate that alterations of Na+ concentration may differentially influence the effect of agonists on phosphoinositide hydrolysis.
SODIUM MODULATES CYCLIC AMP PRODUCTION IN RAT BRAIN SLICES. X. Li, L. Song*, and R.S. Jope, Departments of Psychiatry and Pharmacology, University of Alabama at Birmingham, AL 35294.

Reduction of the sodium concentration from 120 mM to 5 mM in the incubation media dramatically increased the basal cyclic AMP concentration in rat brain slices, with or without forskolin. This inhibition of forskolin effect by low sodium was not altered by changing the calcium concentration or adding EGTA in the incubation media. Isoproterenol and norepinephrine (NI) significantly stimulated cyclic AMP production in slices from rat brain cortex, hippocampus and striatum. Reduction of sodium did not change isoproterenol-stimulated cyclic AMP, but significantly enhanced NE-stimulated cyclic AMP production. However, in low sodium, the stimulatory effect of norepinephrine was significantly increased at 1 mM to 100 mM, and its inhibitory effect was reduced by 50% or more.

These results suggest that sodium modulates cyclic AMP production, possibly by more than one mechanism. Phosphodiesterase inhibition is not likely to be involved and the effect is calcium-independent. Present of NE-stimulated cyclic AMP by low sodium may be indirectly mediated by activating PI hydrolysis. The stimulatory effect of excitatory amino acids is independent of sodium concentration, whereas blockade of quinase-activated cyclic AMP production indicates a regulatory effect of sodium in neuronal activity.


It is known that PGE2 stimulation of peptide release (LHRH) from the median eminence (ME) is mediated by the cAMP pathway, and a short pre-treatment with copper (Cu) markedly amplifies this release process. Since in many tissues stimulation of cAMP accumulation is accompanied by cAMP efflux, we considered the possibility that Cu-amplified PGE2 action is a result of attenuation of cAMP efflux. When rat ME were incubated in vitro, PGE2 induced a rapid (<2.5 min) and sustained (15 min) cAMP efflux, the degree of which was consistent with PGE2 stimulation of PGE2 receptors. By 5 min exposure to 10 μM PGE2, efflux was 58% of basal (100%) and it accounted for 12% of the total (tissue + medium) cAMP. A 5 min pre-treatment with Cu (40-4800 μM) decreased and eventually abolished the stimulation of PGE2 efflux, indicating a moderate 37% increase in content which was not altered by Cu. The size of the functional pool of cAMP is very small relative to the total cellular cAMP and hence, changes in stimulated cAMP-efflux are not reflected in the tissue content. Since Cu amplies PGE2 stimulation of LH-RH release, these results are consistent with the Cu attenuation of cAMP efflux as a mechanism for amplification of PGE2 action mediated by the intracellular cAMP pathway.

ATenosine-cAMP EFFECTS ON FURSHOLIN-INDUCED CYCLIC AMP PRODUCTION IN ISOLATED RAT STRIATAL NEURONS. P. Jope*. The University of Alabama at Birmingham, Birmingham, AL 35294.

Forskolin-induced increases in intracellular cAMP production in isolated rat striatal neurons are reduced by theophylline, which inhibits phosphodiesterase activity. This reduction is more pronounced with forskolin than with the adenylate cyclase activator, 8-bromo-cAMP (8-Br-cAMP). A recent study has shown that adenosine (Ado) induces a cAMP-dependent inhibition of forskolin (Fos)-induced cAMP accumulation in rat striatal neurons. In order to determine the contribution of these effects to the overall inhibition of forskolin-stimulated cAMP accumulation in vitro, we have examined the effects of forskolin and 8-Br-cAMP on forskolin-stimulated cAMP accumulation in the presence and absence of Ado. Forskolin-induced cAMP production was reduced by 32% in the presence of 0.1 mM Ado. Although, Ado-induced inhibition of forskolin-stimulated cAMP production is similar to that observed with 8-Br-cAMP, forskolin-induced cAMP production was only slightly reduced in the presence of 8-Br-cAMP. These results suggest that forskolin-induced cAMP accumulation is not significantly reduced by Ado or 8-Br-cAMP.

A COMPARISON OF THE REGULATORY PROPERTIES OF STRIATAL AND CORTICAL FORMS OF ADENYLATED CYCLASE. LA. DOKAS AND S.M. TING*, Departments of Neurology and Biochemistry, Medical College of Ohio, Toledo, OH 43695.

This study was undertaken to compare the properties of rat striatal and cortical forms of adenylyl cyclase. Enzyme activity was measured in the lysed mitochondrial fraction with a radioisotope assay as described by Gil and Wolfe (JEP 22: 808, 1968). Although basal enzyme activity (5000 pmoi cAMP/min/mg protein) is the same in both preparations, the striatal form is more responsive to forskolin and adenosine 3’-5’-O-(dithiophosphate) (f8-fold stimulation at 10 μM) than is the cortical form (4-fold stimulation). Conversely, cortical adenylyl cyclase activity is stimulated more by GTP. In both cases, 10 μM GTP limits maximal forskolin stimulation to 60% of that seen with forskolin alone. Cholinergic agonists inhibit adenylyl cyclase activity in both brain regions in the order oxotremorine > acetylcholine > carbachol. Much inhibition is seen in response to acetylcholine in the cortex. Although acetylcholine inhibits the striatal enzyme equally, carbachol, less inhibition is seen with the cortical form in the presence of forskolin. The general muscarinic antagonist atropine blocks the effect of 10 μM acetylcholine in both striatum and cortex, while a more selective antagonist tiotidine has little effect at concentrations up to 10 μM, suggesting a high-affinity, tiotidine-insensitive receptor (m2 or m4) is involved. [D-Trp7,2-Cys9]enkephalin inhibits both forms of adenylyl cyclase in a concentration-dependent manner. This indicates significant differences in interactions among subcomponents of the adenylyl cyclase complex in striatal and cortical membranes. Supported by grants from NIH (NS 23596) and the Ohio Department of Aging.
538.9 CANNABINOIDs AND AMINOALKYLINDOLES BIND TO COMMON RECEPTORS TO INHIBIT ADENYLYL CYCLASE IN BRAIN. S. R. Childers, M. A. Pedeco, and S. J. West. Dept. Physiol. and Pharmacol., Bowman Gray School of Medicine, Winston-Salem, NC 27103, and Sterling Research Group, Sterling Drug Inc., Rensselaer, NY 12144.

Both cannabinoid (Cn) and aminoalkylindoles (AAI) bind to specific G-proteins which inhibit adenylyl cyclase receptors as demonstrated by radioactive binding, inhibition of isolated smooth muscle contractions and GTP-dependent inhibition of adenyl cyclase in rat cerebellar membranes. Receptor binding assays have demonstrated that both Cn and AAI bind to common receptors. The current experiments support this conclusion by examining effects of both Cn and AAI on adenyl cyclase (AC) in brain. In rat cerebellar membranes, both AAI agonists and the potent Cn enantiomer inhibited AC with the same efficacy. The IC50 values for levo- and the most potent AAI agonists in inhibiting AC were 0.1-0.3 mM. The dose-response curves of both Cn and AAI-inhibited AC were shifted to the right by an AAI antagonist. The regional distributions of Cn and AAI-inhibited AC in rat brain were identical, with maximal inhibition occurring in striatum and cerebellum. In cerebellum, no additivity in inhibition occurred when maximally inhibitory concentrations of both Cn and AAI agonists were added in the same assay tube. Both AAI agonists and Cn inhibited AC levels in intact cultured rat cerebellar granule cells to the same extent, and these inhibitory actions were blocked by the AAI antagonists. Also, AAI-inhibited cyclic AMP levels were blocked by treatment of cells with phosphodiesterase inhibitors. These data suggest that Cn and AAI bind to common G-protein-linked receptors which inhibit AC in rat brain membranes and in cerebellar granule cells.

538.11 MEATONIN ACTS VIA TWO G-PROTEINS TO INHIBIT ADENYLYL CYCLASE. P. J. Morgan and P. Barrett. Roswell Research Institute, Aberdeen, Scotland, UK, AB2 9SB. Melatonin has been localized on the cells of the mammalian retina and pineal gland (PT), and have been shown to couple through inhibitory G-proteins to adenylate cyclase (Morgan et al., J. Biol. Chem. 264, 1989). The inhibitory effect of melatonin on intracellular levels of cAMP has been seen in several studies with forskolin. Borella pterus tonic (IAP) has been used to investigate the nature of the G-protein coupling between the melatonin receptor and adenylate cyclase.

Primary cultures of optic PT cells were exposed to different doses of IAP (5-50,000 ng/ml) for 16 h, and the effect on the inhibition of forskolin-stimulated APC formation was measured, as described previously (Morgan et al., 1989). IAP attenuated melatonin's inhibitory effect at 10 ng/ml reaching a maximal effect by 100 ng/ml, yet even at this concentration and higher the melatonin response was not completely abolished, with significant (p<0.05) inhibition still attainable. This result indicates that both an IAP-sensitive and IAP-insensitive G-protein are linked to the melatonin receptor.

Using [3H]-Mea, IAP (20 ng/ml) was shown to catalyse the AEP-ribosylation of a 41 K protein in membranes, separated by 125I-iodination, confirming the presence of IAP-sensitive G-protein (G1) in the PT. Furthermore, in membranes prepared from PT cells, pre-treated with IAP (0.5 ng/ml), the subsequent radiolabling with [3H]-Mea was reduced.

The binding of [3H]-3-iodoindole (1.1 nM) to PT membranes is regulated through G-proteins. IAP (0.1-20 ng/ml) attenuates 3H-ML binding to PT membranes in dose-dependent manner, reaching a maximum effect of 20% inhibition. GTP (1 mM) also attenuates binding by 40%, and in combination with IAP this effect is additive. Both of these changes occur through G-protein regulated alteration in melatonin receptor affinity, but IAP alters this affinity through covalent modification of a G-protein, whereas GTP acts via a reversible binding reaction. As IAP only inhibits 20% of 1-ML binding, this indicates that not all G-proteins linked to the melatonin receptor are IAP-sensitive G. This is emphasized by the fact that GTP can still bind further up after IAP treatment. These results, confirm that both an IAP-sensitive and an IAP-insensitive G-protein must link the melatonin receptor to adenylate cyclase.

538.12 LOCALIZATION OF ADENYLYL CYCLASE AND GLUCOSE TRANSPORTER IN RAT BRAIN USING [125I]-LABELED DERIVATIVES OF FORSKOLIN. N. M. Angell, K.B. Scannell, A. Laurence, D. Simpson, and E.B. De Sousa. NIDA/ARC, Baltimore, MD 21204; FDA and NIDDK, Bethesda, MD 20892.

Two iodinated derivatives of forskolin have been synthesized that show differential specificity for adenyl cyclase (6-[125I]-Fsk, 2200 Ci/mmol) and for the glucose transporter (7-[125I]-Fsk, 2200 Ci/mmol). These iodinated compounds have been used to locate forskolin binding sites in rat brain sections using autoradiography. The distribution of both 6-[125I]-Fsk and 7-[125I]-Fsk binding sites was similar to that previously reported for [H]-forskolin. Highest densities were noted in caudate putamen, nucleus accumbens, olfactory tubercle, substantia nigra (reticulata), superior colliculus (superficial), globus pallidus, and, as previously described, the medial septum and nucleus basalis of Meynert.

Binding in cerebral cortex and hippocampus was less intense, but while binding in cerebral cortex was uniform, binding in hippocampus was enriched in the molecular layer of dentate gyrus and pyramidal cell layer of regions CA2 and CA3. The binding of 6-[125I]-Fsk was inhibited by forskolin but not by 1,9-dideoxyforskolin, consistent with these sites being associated with adenylate cyclase. Agents that inhibit forskolin binding to the glucose transporter, such as cytochalasin B and D-glucose decreased 7-[125I]-Fsk binding but did not decrease 6-[125I]-Fsk labeling. Thus it appears that 6-[125I]-Fsk binds exclusively to adenylate cyclase and can be used as a specific ligand to measure adenylate cyclase binding sites. In contrast, 7-[125I]-Fsk binds to sites that are associated with the glucose transporter binding sites in brain. These novel derivatives can be used to study the differential localization of enzymes involved in signal transduction and proteins involved in glucose utilization.


Forskolin-induced, activation of adenylate cyclase was used to explore dopamine (DA) stimulation and inactivation of adenylate cyclase accumulation in rat brain slices from striatal and limbic regions. Stimulation of cyclic AMP production was measured by the conversion of [3H]-adenine to [3H]-cyclic AMP. In striatal and limbic regions, the EC50 value for SKF38393-induced D1-stimulation of adenylate cyclase was 1 x 10^-8 M, which is consistent with published values from other methodologies. The selective D1 agonist SC54390 inhibited this stimulation. The D2 agonist setron inhibited the D1-stimulated increase in cyclic AMP accumulation in the striatal region by 50%. The D2 antagonist, prazosin blocked this accumulation which indicates that the inhibition of adenylyl cyclase by PRT is a D2 receptor-specific event. The D2-mediated increase in cyclic AMP accumulation was detected in the brain areas that have the highest density of D2 receptors. The D2-mediated inhibition of cyclic AMP accumulation was seen in the caudate but not the putamen. The assay system characterized here enables efficient study of D1- and D2-receptor interaction and modulation.
Furthermore, a 10% increase in the concentration of intracellular calcium (Ca++) was observed in the presence of 10^{-8} M Ca++, which is consistent with previous reports. The observation of a significant increase in intracellular calcium levels suggests a potential role for these receptors in mediating calcium-dependent processes.

In conclusion, the experiments presented in this study provide evidence for the involvement of specific receptors in the regulation of intracellular calcium levels. Further studies are needed to elucidate the mechanisms underlying these processes and to determine the physiological relevance of these receptor-coupled events.
539.1


Amphetamine (A) (20 mg/kg, IP) in combination with iprindole (I) (100 mg/kg, IP) is known to produce, similar to repeated high doses of A or meth-A, a long-lasting depletion of DA in certain brain areas, particularly, striatum. Last year (SN Abst. #597, 1989, L. Wichtlin et al) we reported increases in apomorphine (APO) induced behaviors in male rats (locomotor activity) 3-10 weeks after A. We now report [1] replication of these behavioral data; [2] confirmation of DA depletion (at 4 weeks) in striatum (45%) but not n.accumbens (6%); [3] increases in APO induced responses in Bmax (but not Kd) for sulpride sensitive [3H]priporeidol binding to striatal D2 DA receptors (p < .05). Furthermore, the peptide cyclo(eugly)CGL, a neuromodulator which we showed down-regulates DA receptors in many different paradigms, significantly reduced APO-induced behaviors in A/I rats and reversed the increases in D2 DA binding (fimA/mg prot: control=74, A/I=266; A/I+CGL=216, p < .05). All may represent a useful model of long-term D2 DA up-regulation. (Supported by grants from the VA, Scottish Rite Schizophrenia Res Pgm-NMI, & NIH [NS26499].)

539.2


Long term administration of neuroleptics is frequently associated with the development of receptor supersensitivity. We report to GTP of striatal adenylate cyclase activity in membranes from D1, D2 and D1+D2+ D2 receptor antagonists. G-proteins in the coupling of rat striatal dopamine receptors to adenylate cyclase activity of up-regulated receptors, SCH-23390, haloperidol, SCH-23390 + haloperidol and saline was administered to male Wistar rats over a 21 days period. Our results show that, besides the expected variation of dopamine and D1 agonist (+SFP-28393) stimulated adenylate cyclase activity induced by up-regulated receptors, also the response of the enzyme activity to TPF alone is modified. These results suggest that the increased sensitivity of G-proteins to GTP may participate in the mechanisms of induction of receptor up-regulation and be involved in their enhanced functional response.

539.3

EFFECTS OF CHRONIC TREATMENT WITH LOW DOSES OF L-SULPIRIDE ON DOPAMINE RECEPTOR AND D-ADRENERGIC RECEPTOR FUNCTION IN RAT STRIATUM AND FRONTAL CORTEX. C. Missale, S. Sigala, P. Rizzolatti, A. Fargione and P.F. Spina. Inst of Pharmacol Exp Ther, Sch of Med, Univ of Pavia, Italy.

There is accumulating evidence that L-sulpiride has antidepressant activities when administered at low doses. Down regulation of D-adrenergic receptor function in the frontal cortex is a well documented adaptive response to chronic administration of antidepressants. On these basis we studied the responses of striatal and frontal cortex DA receptors and B-adrenergic receptors to chronic administration of low doses of L-sulpiride. Male Sprague-Dawley rats were treated with 2 mg/kg (i.p.) L-sulpiride twice a day for 21 days and killed by decapitation 8 days after the last injection. The results showed that the function of striatal D1- and D-2 receptors is decreased by L-sulpiride administration, suggesting that at low doses L-sulpiride preferentially blocks DA autoreceptors controlling DA release. L-sulpiride also induced a selective desensitization of corticale B-receptors; this effect was not detectable in the striatum which contains a high density of these receptors, but lacks norepinephrine (NE) innervation. Cortical ME terminals may be endowed with D2 receptors controlling ME release and blockade of this endogenous ME inhibitory modulation may be involved in the antidepressant effects of L-sulpiride.

539.4


Rats, chronic neuroleptic agents, develop an increased stereotyped behavioral response to a subsequent challenge with the dopamine (DA) agonist apomorphine (APO). DA receptor proliferation may be responsible for this behavioral hypersensitivity (BH). We have studied chronic neuroleptic paradigms to examine the relationship between BH and DA receptor proliferation. Animals were treated for two months with haloperidol (3.0 mg/kg), thioridazine (20 mg/kg/20 mg/kg), or clozapine (3 mg/kg). Four days following the last treatment the animals were challenged with APO and 2 days later they were sacrificed. The striata were examined for alterations in D-2 receptor number and affinity. Quantitative autoradiography, AMAN, LI, SCOP, and THIO cotreatments significantly attenuated the development of BH. SCOP cotreatment also attenuated the development of BH, however, failed to attenuate HAL-induced BH. AMAN and LI cotreatments prevented the D-2 receptor proliferation normally induced by HAL while SCOP, THIO and CLZ cotreatments did not. Therefore, D-2 receptor proliferation can exist in an animal that does not exhibit BH suggesting that other factors in addition to D-2 receptor proliferation may participate in the expression of BH. D-2 receptor proliferation is therefore permissivel for the expression of BH.

539.5


Male Sprague-Dawley rats were implanted s.c. with Alzet osmotic minipumps containing either naltrexone (NTX) or saline (SAL). Half of each group was then given free access to the potent opiate etonitazene (ET; 2 mg/kg) or saline (SAL) for 12 days. Consumption of the SAL-implant rats gradually rose from 30 to 60 ml/day, but this effect was blocked by NTX. Striatal synaptosomes showed increased high affinity binding of [3H]-raclopride to D2 receptors from SAL animals who ingested ET, with no significant change in Bmax. These changes were abolished by NTX. ETA receptor binding was unchanged. Computer modeling was consistent with naltrexone acting as a partial agonist in the D2 binding following chronic ET ingestion representing 10-20% of the D2 receptors having a 50-100-fold higher affinity. Since antagonists were used for binding and competition, it is unlikely that the modulation of binding is due to changes in receptor/G-protein interaction. (Supported by USPHS DK 39328, DA 06539 and ONR)

539.6

CHANGES IN BASAL GANGLIA DOPAMINE (DA) SYSTEMS AFTER NEONATAL INTRAVENTRALTAL 6-HYDROXYDOPAMINE (6-OHDA) INJECTIONS. B.S. Neal and J.N. Joyce. Departments of Psychiatry and Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

It is known that early lesions of DA systems result in different behavioral effects compared to adulthood lesions. Nonselective damage to DA systems in the early postnatal period results in enhanced responses to D1 agonists, but not to D2 agonists. Thus, there are clear differences as a function of age, depending upon the nature of the system lesioned. To selectively destroy the DA input to the putamen (striosomes) compartment, while leaving that to the matrix relatively intact, we infused 6-OHDA into striatum (4 mu per striatum) on day of birth (P0) or postnatal day (P1). The rats were supersensitive to the behavioral effects of D1 agonists and subsensitive to the effects of D2 agonists to adults. When challenged to adulthood, DA receptor changes within the striatum could account for these effects. Quantitative autoradiography on coronal sections of tissue from rats treated bilaterally with 6-OHDA lesions revealed a significant and patchy loss (30-50%) of presynaptic DA uptake sites (TH-mazindol), with greater losses in the dorsomesial and dorsolateral regions, there were changes in the density of D2 sites (TH-spiroperidol); however, there was a significant and heterogeneous loss (10-20%) of D1 sites (TH-SC29390). Decreases (40-50%) in the number of postsynaptic receptor (TH-naloxone) patches were also apparent. A second set of neonatally-lesioned rats was killed on P7 (before the patch/matrix organization is obscured) in order to determine if there was a selective loss of D1 receptors in the patch compartment. Preliminary results suggest that this is the case. Tyrosine hydroxylase immunocytochemistry is also being used to examine the intrinsic dopaminergic innervation of the directed DA inputs in these animals. (Supported in part by USPHS Grant MH43852.)
355.7


It has been demonstrated that chronic administration of selective neurotransmitter antagonists produces an increase, or up-regulation, in the density of the specific receptor [(Bmax) A novel D-1 antagonist, SCH 39166 ([trans-](6S,8R,9S,13b)hexahydro-3-chloro-2-hydroxy-N-methyl-5-H-benzo[d]-[n]aphtho[2,1b]benz[e][6]-de-[j]nicotine)1] binding sites in a number of tissues, including the brain. A novel D-1 antagonist, SCH 39166 ([trans-](6S,8R,9S,13b)hexahydro-3-chloro-2-hydroxy-N-methyl-5-H-benzo[d]-[n]aphtho[2,1b]benz[e][6]-de-[j]nicotine)1] was examined for its ability to produce such a change in Bmax when administered chronically. Adult thomus monkeys received daily i.v. injections of either vehicle or SCH 39166 (3, 12 or 48 mg/kg) for three months. The caudate nucleus and putamen were dissected and the D-1 and D-2 receptors were analyzed for changes in their affinities (Kd) and densities. In the putamen, a significant increase in Bmax was seen for all three doses of SCH 39166 (58, 92 and 100% above control, respectively, p < 0.05, Duncan's multiple range test), while in the caudate nucleus only the 12 and 48 mg/kg doses produced a significant up-regulation when compared to vehicle controls (50 and 65%, respectively). No significant increase in the density of D-2 receptors was observed in either region.

Similar studies were performed in male rats that had received 50 mg/kg SCH 39166 orally for 7 consecutive days. Analysis of striatal membranes from these groups indicated that SCH 39166 produced a significant increase in the density of D-1 receptors (53% over control animals), without affecting the density of D-2 receptors.

These data demonstrate that in vivo, SCH 39166 is capable of crossing the blood brain barrier and binding to D-1 receptors in rodents and primates. Further, the selective up-regulation of these receptors suggests that, in vivo, SCH 39166 binds to D-1 receptors without significant interaction at D-2 sites.

359.9

OPPOSING EFFECT OF CHOLECYSTOKININ OCTAPEPTIDE ON SPECIFIC DOPAMINE D2/D1 TYPE BINDING SITES IN RAT BRAIN. J.M. King, C Main, L. Stagg, T.N. Chase, ETR. NINDS, Bethesda, MD 20892.

Blockade of either dopamine (DA) D1 and/or D2 receptors induces catalepsy. Cholecystokinin-octapeptide (CCK8) affects central dopaminergic function, possibly by modulating DA interactions at both D-1 and D-2 receptor sites. The present study was designed to explore the effects of acute intrastriatal injection of CCK8 on D-1 and D-2 receptor mediated catalepsy and the binding capacities of DA receptors at the time of maximal behavioral response. Over a period of 3 minutes, CCK8 (50-800 ng) was injected into each striatum through surgically implanted cannulae. For catalepsy induction, rats received either the D-1 antagonist SCH 23390 (0.5 mg/kg sc), the D-2 antagonist raclopride (RAC, 2.5 mg/kg ip), or saline. Catalepsy was scored for the next 2 hours. Other rats were sacrificed 20 min after antagonist injection, when catalepsy peaked. Brains were removed and P2 membrane fractions prepared for sc,avp receptor assay. CCK8 significantly diminished SCH induced catalepsy but enhanced RAC induced catalepsy. Given alone, CCK8 did not affect motor behavior, but did increase the number of available receptors of both subtypes. The presence of SCH or RAC alone, reduced the number of respective binding sites available; intrastriatal CCK8 increased the number of available D-1 and decreased the number of available D-2 binding sites. The opposing effect of CCK8 on D1/D2 receptor binding and catalepsy may provide a mechanism for the modulation of the functional interaction of the dopaminergic system by CCK8 either directly or via one of its metabolites.

359.11

KINETICS OF NICOTINE BINDING TO BRAIN TISSUE AFTER CHRONIC NICOTINE INFUSION. B.S. Bhat, B.L. Turner*, R.L. Marks and A.C. Colling, Sch. of Pharmacy, Inst. for Behav. Genetics, Psychology Dept., Univ. of Colorado, Boulder, CO 80302.

The basic model for desensitization of the nicotinic receptors from Torpedor olomira can be viewed as two conformational states of the receptor having differential affinities for the agonist. Although less studied, it has been suggested that a similar situation occurs with the nicotine binding sites in the brain since receptors in fast (fs) and slow (s) phases of association binding can be detected. Chronic nicotine treatment increases the number of [3H]nicotine binding sites in rodent brain sites and in decreased sensitivity to nicotine. A conformational shift from a low affinity curve to a higher affinity desensitized form may be responsible for this adaptive effect. Thus, chronic nicotine treatment should cause a shift in the ratio of Bs to Bf binding. C57BL/6 mice were continuously infused with saline or 0.5, 1, 1.3, 6.9 ng/kg/h nicotine for 7 days. The kinetics of [3H]nicotine binding to brain membranes obtained from these mice revealed biphasic association and a monophasic dissociation kinetics. Chronic nicotine treatment did not alter the Bs/Bf ratio. Since the rate of desensitization of the receptors is not known, additional data are necessary to test whether the Bs/Bf ratio is altered in vivo. Supported by DA-00194 and DA-00116.

355.8

BRAIN SIGMA AND DOPAMINE RECEPTORS ARE NOT MODULATED BY CHRONIC D-PENTAZOCINE ADMINISTRATION IN RATS. A.D. Weissman and E.B. De Souza. Neurobiology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224.

Schizophrenia has been associated with alterations in the regional brain densities of D-1 and D-2 dopamine receptors. Recent studies have suggested that drugs with high affinities for the D-1 receptor may produce psychotomimetic effects by modulation of dopamine receptors. Long-term treatment of animals with D-2 drugs can regulate the number of D-2 binding sites. We hypothesized that chronic administration of a drug such as D-pentazocine, with high affinity and specificity for D-1 receptors, could alter the number of D-2 binding sites and indirectly affect basal dopamine release. This, in turn, should modulate the number of D-2 receptors. Rats (n = 6/group) were implanted s.w.t. with mini osmotic pumps to deliver either D-pentazocine (10 mg/kg/day) or saline for 4 weeks. Saturator studies using [3H]Yohimbine or [3H]Haloperidol in the presence of 50 nM spiperone to label D2 and D1 sites, respectively, revealed no significant changes in the Kd of either site in older brain regions of drug treated animals. The inability of the specific compound D-pentazocine to alter D-2 receptors differentiates this drug from compounds such as D-3 and DTG that up-regulate D-1 binding, and from haloperidol, which down-regulates D-1 sites. Unlike D-pentazocine, the compounds that are able to regulate D-2 receptors bind to multiple sites. This suggests that chronic interaction with the haloperidol-sensitive site alone may not be sufficient to modulate D-2 dopamine binding.
540.1

IN VIVO RELEASE OF ACETYLCHOLINE IN THE BASAL NUCLEAR COMPLEX FROM NEURONS OF THE PONTOMESENCEPHALIC TRIGEMION (PMT) S. Consolo, R. Bertorelli*, G.G. Forloni and I.G. Butcher*, Istituto "Mario Negri", 20157 Milan, Italy and "University of California, Los Angeles, CA 90024-1563, USA.

In an attempt to determine possible interactions between the basal forebrain (NF) cholinergic complex and the PMT cholinergic neurons, we measured the acetylcholine (ACh) release in vivo from the NF, both alone and in combination with lesion and pharmacologic manipulations. The ACh release was calcium dependent and it was increased by scopolamine (SCOP) (0.5 mg/kg s.c.). The rise in ACh release induced by SCOP (a) persisted in the presence of quisqualate lesions of the NF complex (b) was blocked by tetrodotoxin infusion, and (c) appeared to be controlled by presynaptic muscarinic receptors on PMT axon terminals. Thus, the PMT cholinergic complex might influence cortical ACh release, in part at least, by means of serial-order cholinergic-cholinergic interactions in the basal nuclear complex.

540.2


The effects of a novel M3 receptor agonist, AF102B (FKS-508; cis-2-methylisopropyl (1,3 - oxathiolane - 5') quinuclidine, on the central cholinergic system in vivo were evaluated by determination of acetylcholine (ACh) content in the rat brain after microwave irradiation and by measurement of ACh release with microdialysis perfusion in freely moving rats. Intraperitoneal administration of AF102B resulted in a significant decrease of ACh content in the brain, while AF102B produced an increase of in vivo ACh release. The present results suggest that ACh content in the brain after treatment with muscarinic agents may be related to the changes of ACh release, in which both M1 and M2 muscarinic receptors may be involved.

540.3

MODULATION OF HIPPOCAMPAL ACETYLCHOLINE RELEASE BY NMDA RECEPTORS M.G. Slovanny* and G. Pepe, Department of Pharmacology, University of Florence, Florence, Italy.

Evidence has been accumulating that the cholinergic and glutamatergic systems of the hippocampus are involved in cognitive processes. While the cholinomimetic action of nicotinic agonists on the cholinergic and glutamatergic systems in the hippocampus is well known, little is known of the possible interactions between the two neurotransmitter systems. The aim of the present study was to investigate the effect of the modulation of hippocampal muscarinic receptors on the release of ACh in freely moving Wistar male rats by means of a transversal microdialysis probe implanted in the proximity of the CA1 region in the dorsal hippocampi. Perfusion of the microdialysis probe with 500 μM NMDA dissolved in Ringer solution brought about a decrease of ACh release compared to the mean of three basal control values (--48 %, P<0.05, Duncan test). A decrease (-- 45 %, P<0.05) was also observed following perfusion with the NMDA antagonist 2-amino-7-phosphonoheptanoate (AP7). On the contrary, i.c.v. injection of AP7 (10 μg/ rat) was followed by an increase in ACh release (+79 %, P<0.05). These findings indicate a complex modulation of the cholinergic system by NMDA receptors. Supported by C.N.R. grants.

540.4

ACETYLCHOLINE RELEASE IN THE HIPPOCAMPUS, CORTEX AND STRIATUM OF RATS CORRELATES WITH LOCOMOTOR ACTIVITY: AN IN VIVO MICRODIALYSIS STUDY. J. DAY, G. DAMMA, R.C. DUBBER, Department of Pharmacology, Univ. of Michigan, Ann Arbor, MI 48109, USA.

Simultaneous measurements of locomotor activity and dopamine concentrations of acetylcholine (ACh) and choline (Ch) in the dorsal hippocampus, frontal cortex, and dorsal striatum of rats were made under three conditions: 1) drug-free, after the administration of vehicle (H2O); 2) after administration of the dopamine receptor antagonist, haloperidol (0.5 mg/kg); and 3) during the change from light to dark in the rats' daily cycle. Vehicle injections increased ACh concentrations in the cortex and hippocampus, but not in the striatum. Scopolamine stimulated locomotion and increased extracellular concentrations of ACh to 404% (striatum), 124% (hippocampus) and 1435% (cortex) of basal values. Ch concentrations in the hippocampus and cortex were lowered slightly by scopolamine; Ch in the striatum was transiently increased by this drug. Within 20 minutes after dark, ACh increases of 58%, 77% and 169% were measured in the striatum, cortex and hippocampus, respectively. Locomotor activity also increased during this time. Dark exposure did not change Ch in any area. Significant positive correlations between ACh release and locomotor activity were found for each brain region under all three conditions. These results indicate that cholinergic transmission can undergo regionally selective changes in response to environmental and pharmacological stimuli.

540.5

AMPHETAMINE-INDUCED INCREASES IN STRIATAL ACETYLCHOLINE RELEASE AS MEASURED BY MICRODIALYSIS ARE NOT DEPENDENT ON NIGROID STRIATAL Dopamine. R.J. MANUEL, G.D. NELSON*, P. ROSENBERG*, and A. BÜRKELUND. University of Lund, Dept. of Medical Cell Research, Biologigatan 5, 223 62 Lund, Sweden.

One of the most consistent findings in the study of striatal dopamine (DA) receptor pharmacology is the inhibitory control by D2 receptor stimulation of striatal acetylcholine (ACh) release in vivo. The present study was undertaken to characterize the in vivo dopaminergic control of ACh release in the rat striatum. In preliminary experiments, we observed a consistent 100-200% increase in striatal ACh release in response to 5 mg/kg systemic amphetamine (AMPH, ip) but no effect when AMPH was administered locally in the probe (10 μM AMPH, 3 μM scopolamine, 15 μM amphetamine). Since the systemic data are in direct contrast to many reports using the in vivo slice technique, an experiment was undertaken to determine whether the effect was dependent on striatal DA. Eleven rats were administered unilateral 6-hydroxodopamine lesions with 5 receiving desmethylergocryptine to protect nonlesioned striatal projections. All rats were implanted with bilateral microdialysis probes in the striatum (AP 0.7, mm LAT -2.6, DV -6.0, from bregma and dura). There was no effect of lesion on either baseline or AMPH-induced striatal ACh release with systemic AMPH: Lesioning a 100-200% increase compared to baseline levels. A preliminary experiment (n=2) utilizing an extremely ventrolateral striatal placement of the dialysis probe in intact rats also indicated a large increase in striatal ACh release in response to systemic AMPH. These data suggest that local striatal release of DA is not responsible for the observed effect of AMPH on ACh release and there is no differential dopaminergic control of striatal ACh in these striatal subregions. Thus, these multisynaptic pathway, perhaps that can be influenced by the highly nigrostriatal DA projection, must be responsible for the effects reported here.

540.6


Neurotensin (NT) immunoreactivity and receptors are present in the striatum, a region enriched with dopaminergic and cholinergic innervation. The present study determined whether NT alters the activity of striatal cholinergic interneurons and if nigral DA neurons modulate this effect. NT (10 μM) increased K+-evoked (50μ) but not basal, ACh release from striatal slices. The effect was concentration-dependent and tetrodotoxin-insensitive. In the presence of the dopamine antagonist sulpiride (50 μM), the effect of NT 10 μM was further enhanced by an additional 40%. In slices from 6-OHDA lesioned rats, in which striatal ChAT activity was not changed, but basal and evoked ACh release were increased (33% and 40%, respectively), the effect of NT on evoked ACh release was similar to control (4% increase). In addition, in lesioned animals, sulpiride (50 μM) did not alter evoked ACh release, nor did it augment the effect of NT (10 μM) alone. These results suggest that in the striatum, NT regulates ACh release by a direct action on cholinergic elements. (Supported by NSC, Canada and FRSQ, Quebec).

Sodium dependent high affinity choline uptake levels are increased following i.p. injections of scopalone or pentylenetetrazol, and decreased by pentobarbital. This study examined the effects of these pharmacologic manipulations on two other measures of cholinergic activity, ACH release and the number of high affinity choline uptake (HACU) sites. Changes in release of ACH were determined by in vivo microdialysis. Six 5-minute microdialysate samples, from the hippocampus and overlying cortex, were collected to obtain baseline levels prior to drug injection and an additional twelve successive dialysate samples were collected after drug injection. Levels of choline and ACH were assayed using HPLC with electrochemical detection. One hour prior to the determination of HACU binding by hemicholinium-3, each rat was given an injection of one of the three drugs. A correlation between the kinetics of ACH release and HACU binding following drug injections that alter the activity of the cholinergic system can be determined using this technique. Supported by NSF BNS 88-07010 and NIH NS 20471.

540.7

540.9

540.11

540.12

540.10

540.8

EFFECT OF THE VAGUS NERVE STIMULATION ON ACETYLCHOLINE RELEASE ASSESSED BY MICRODIALYSIS IN RABBIT BRAIN. E. Messamore, N. Ogane, E. Giacobini and E. Williams*. Dept. of Pharmacology, Southern Illinois Univ. Sch. Med. Springfield, IL 62797, USA.

Heptagstigmine [heptyl-physostigmine(MF-201), Mediolanum Farm., Milan, Italy] is a new derivative of the carbamate acetylcholinesterase (AChE) inhibitor physostigmine (Phy) with lower toxicity and longer inhibitory action on brain AChE than the parent compound. Following single dose administration of 5 mg/kg i.m. an 80% inhibition of brain AChE is reached at 60 min; at 360 min inhibition is still 67%. Increases in acetylcholine (ACh) and homovanillic acid (HVA) metabolites are seen in all brain regions with a maximum increase of 60% occurring in striatum 2h after injection. Local AChE inhibition by Phy as well as MF-201 administered via microdialysis probe elevates ACh recovered from striatum in a dose-dependent manner. In contrast to the ACh elevating effect of AChE inhibition observed in the aforementioned studies, systemic administration of MF-201 (5 mg/kg) decreases ACh recovered by microdialysis from the striatum of freely moving rats; ACh falls to 84% of its baseline value 2 hours after i.p. injection. Microdialysis permits recovery of ACh from the extracellular space, while assay of brain homogenate reflects extracellular as well as intracellular compartments. MF-201 decreases ACh measured by microdialysis yet increases ACh measured by AChE. Therefore ACh elevation in brain homogenate following a high level of AChE inhibition reflects a predominantly intracellular drug effect.
ACETYLCHOLINE III

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

540.13

DOSE-TIME EFFECT OF ETHEROCHLORIZIDIN (AF64A) ON CYCLIC AMP (cAMP) LEVELS IN RAT BRAIN: CORRELATION WITH THE REVERSIBILITY OF CHOLINERGIC DISRUPTION AT LOW DOSES OF AF64A


AF64A administration produces a cholinergic dysfunction that may occur in a possible feedback loop, involve noradrenergic mechanisms (Hortonru et al. J. Neurochem. 54: 407-414). Later, whether the second messenger cAMP might reflect these AF64A-Induced catecholaminergic changes was investigated. Animals were treated with various doses of AF64A (0.5, 1.0, and 2.0 mmol/kg) intraperitoneally, and killed by decapitation at 2, 4, 7, 14, 28, and 42 days after such treatment. Hippocampus, striatum, and frontal cortex were rapidly dissected out and tissue fractions were subsequently extracted in 6% Triton X-100 on ice. The extracts were prepared by homogenization, centrifugation, and ultrafiltration with a microfilter. Choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities were also measured in a portion of these fractions. Changes in cAMP, ChAT, and AChE were observed only in the hippocampus, thus indicating region-specific effects of AF64A. By 2 days following AF64A treatment, at all doses tested, there was a dose dependent (23-36%) decrease in hippocampal cAMP levels, respectively. By 7 days, all values returned to normal. Hippocampal ChAT and AChE reductions following doses of 0.25 and 0.5 mmol/ventrcle reached a nadir (25-50%) by 4 and 7 days, respectively, but returned completely to normal values within 14 days following AF64A treatment. ChAT and AChE levels following treatment with 1.0 and 2.0 nmol AF64A/ventricle were reduced significantly by 4 days, and did not recover even 45 days post AF64A. These combined data imply a modulatory role for cAMP dependent mechanisms in the disruption of central cholinergic function induced by AF64A treatment.

540.15

DECREASED CHOLINE ACETYLTRANSFERASE ACTIVITY AND 3H-ACETYLCHOLINE RELEASE IN THE RAT HIPPOCAMPUS FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION

D. Boeckmann and H.J. Metcalf, Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada K7L 3N6

We have previously shown that ACe release in the medial septal/diagonal band complex (MSD) may be stimulated by 35 mM potassium (K+). However, the origin of this ACe release is not known. Electrophysiological and morphological data suggest that the release may be from local collaterals originating from cholinergic neurons projecting to the hippocampus. Inclusion of the cholinergic hippocampal pathway produces a complex of subregions of the hippocampus. Animals were subjected to 1 hour of ischemia, and were allowed to recover for 1, 3, and 5 weeks. ACe histochemistry 1 week following ischemia revealed an absence of swollen ACe-positive fibers in the MSD which by 3 weeks had disappeared, along with a large majority of ACe-positive cell bodies. This loss was still evident at 5 weeks. ACe activity measured in the hippocampal and MSD of non-operated controls was 65.4 ± 7.3 and 137 ± 10.7 nmol/mg protein/hr respectively. When compared to controls, ChAT activity in the hippocampus increased by 80% 1 week following ischemia. In the MSD, ChAT activity decreased by 30% 1 week following ischemia and was decreased by 32% at 3 weeks. This decrease was still present at 5 weeks. In normal animals, 35 mM K+ produced an 11.2 ± 1.9 fold increase in MSD 3H-ACe release. One week following ischemia, 3H-ACe release was the same as that observed in control experiments. At 3 weeks, a 50% decrease in release was observed in the MSD. 3H-ACe release was still decreased at 5 weeks. These results suggest that a portion of cholinergic activity seen in the MSD may be due to local collaterals of cholinergic neurons projecting to the hippocampus. Supported by the MRCC and OMHF.

540.17

EFFECTS OF CHOLEINE AND FETAL SEPTAL GRAPTS ON SOME CHOLINECERGIC PARAMETERS IN FIMBRIA-FORNIX LESIONED RATS


A 2x2 mm cavity was made by suction unilaterally on the surface of right hippocampus, and fimbria-fornix was lesioned in 31 rats under intubation 145 mg/kg anesthia. Two weeks after, the cavity was re-opened and fetal septal grafts were inserted in 15 rats ("septal-grafted") The remaining 16 rats served as the vehicle-treated group. Some rats in each group were given choline in their drinking water (15 mg/ml) for period of 8 weeks by starting 5th week of the grafting. 11th week of the grafting, motor activity of rats in the right hippocampus was measured. 12th week of the grafting rats were killed and their brains removed. Choline acetyltransferase (CAT) activity and 3H-QNB binding were measured in sham-grafted rats, respectively. In choline altered none of these parameters in septal-grafted rats, but restored, greatly, toward normal decreased motor activity in sham-grafted rats.

These results show that long term choline supplementation has no harmful effect on the repairing action of cholinegenic fetal septal grafts, and restores some of behavioural consequence of cholinergic neurons lost.

540.18

IMMUNOHISTOCHEMICAL EVIDENCE THAT A VESAMICOL ANALOGUE REDUCES THE EXPRESSION OF CHOLINERGIC ACETYLTRANSFERASE (CHAT) IN THE RAT BRAIN WITHOUT AFFECTING OTHER NEUROTRANSMITTER-SPECIFIC MARKERS


The compound trans-2-(4-Phenylpiperidino)cyclohexanol (now called Vesamicol) is known to inhibit the storage of acetylcholine in nerve terminal synaptic vesicles. A number of analogues have been synthesized recently and their potential use evaluated on purified synaptic vesicles or Torpedo electric organ (Roger et al, J. Med. Chem. 32: 1217, 1989). We tested the effects of intraecranbroventricular (i.c.v.) administration of one of these drugs, hereafter referred as OD72. Adult Sprague-Dawley rats were divided into control i.c.v. with OD72, 10 nmol, and furthermore, transpedally 7 days after surgery. In OD27-treated rats we found a disappearance of neurons expressing CHAT immunoreactivity in the cholinergic cell body nucleus on the injected side, while CHAT-positive neurons in the brainstem were not affected. Other cholinergic markers (nerv growth factor receptor, acetylcholinesterase) were not changed. In addition, the number of neurons expressing GABA-like immunoreactivity in medullary septum, as well as cells in the locus coeruleus immunoreactive for dopamine-B-hydroxylase (DBH) was unaltered as a consequence of OD27-administration. No alterations of nissl stained cells were observed. These data suggest that OD72 might be a drug capable of reducing CHAT expression selectively in the basal forebrain, without affecting noncholinergic markers like DBH or GABA. [Support: NS 19898]
541.1 COLocalization of 11B-Hydroxysteroid Dehydrogenase and Mineralocorticoid Receptor in Rat Brain. R. P. Sakai, V. Lakshmi, C. M. Howard*, E. F. Bender, Z. Krozovitz, and R. M. Pfaff. The Rockefeller Univ., Dept. of Neurobiology and Behavior, New York, NY 10012; and Medical Research Centre, Prince Henry’s Hospital, Melbourne 3004, Australia.

The activity of 11B-hydroxysteroid dehydrogenase (11-De) which converts corticosterone to inactive 11-dehydro metabolites, may provide a mechanism which enables aldosterone to gain access to Type I (mineralocorticoid) receptors despite the uncoordinated delivery of 100-1000 fold higher concentrations of corticosterone. To further test this possibility, we examined the immunocytochemical localization of Type I receptors and 11-De in rat brain, with antibodies directed against renal mineralocorticoid receptor and hepatic 11-De. Type I positive cells were localized throughout the brain, complementing their known distribution by receptor binding assay. The hippocampus showed the highest Type I receptor density, followed by the cortex, paraventricular nucleus of the hypothalamus and amygdala. 11-De colocalized with Type I immunoreactivity in all brain areas demonstrated to contain 11-De in the hippocampus. These data support the idea that 11-De is important in mediating the expression of aldosterone specific effects at the Type I receptor and further suggest that the action of 11-De in relation to the mineralocorticoid in the brain may be via an autocrine rather than a paracrine mechanism as is believed to occur in the kidney.

541.3 THE ROLE OF ESTROGEN IN THE DEVELOPMENTAL DISTRIBUTION OF CALCIUM CYSTOKININ (CCK) RECEPTORS IN THE RAT BRAIN: A QUANTITATIVE IN VITRO AUTORADIOGRAPHY STUDY. B. D. Richardson, M. Martin, J. S. Jacobowitz, and J. F. MacLusky. Dept. of Reproductive Science, University of Toronto, Toronto, Ontario M5G 1L7.

Quantitative in vitro autoradiography studies have shown sex differences in CCK receptor levels in rat brain. Male and female rats were gonadectomized and implanted (i.e.) after 1 week with either vehicle or 1% estradiol Silastic capsules 3 days prior to sacrifice. Tissue sections (10 um) were preincubated at 30°C for 15 min. with 50 mM Tris-MgCl2 buffer with 0.2% bovine serum albumin, ImMs dl-thriothreitol, and 0.2% bacitracin (PH 7.7) followed by a 1 hr incubation with 0.1 mmol, 11-C,8 in the preincubation buffer. Females showed increased levels of CCK receptors in the hypothalamic ventromedial nucleus (VMN) and the cingulate cortex compared to the males. Estrogen treatment products a significant decrease in CCK receptors in both the VMN and cingulate cortex of female rats, but had no effect in the male rats. These findings are in agreement with Akesson et al. (Neuroendocrinol. 45: 257-262, 1987) who reported that a bolus injection of estradiol 24 hrs prior to sacrifice significantly reduced CCK binding in the VMN in ovarectomized rats. These results suggest a sexually dimorphic distribution of CCK receptors in the rat brain, and in modulating these receptors in the female rat. Supported by MRC Canada.

541.5 THE ORIGIN OF TRANSFERRIN RECEPTORS IN THE DEVELOPING CHICK RETINA. Arnold G. Hyndman, and Sa-Sen Chia. Department of Biological Sciences, Rutgers University, Piscataway, NJ 08855.

Transferrin is a growth factor likely to play a significant role in CNS development. In this study, the appearance of the transferrin receptor (TFR) in the developing chick retina is examined. TFR immunoreactivity is first detectable in the ganglion cells of E (embryonic day) 4 retina. At E10, TFR immunoreactivity is seen in the inner nuclear layer and in photoreceptor cells. In contrast to transferrin, which is characteristic located in the plexiform and nerve fiber regions, and in the outer segments of the photoreceptors, TFR is localized primarily on somas. TFR immunoreactivity in ganglion cells and the inner nuclear layer decreases during the later stages of embryonic development (E5 to hatching), but remains strong in the photoreceptors. In addition to neurons, Müller cells and glia of the optic nerve fiber layer are TFR positive. The TFR positive glia of the optic nerve fiber layer are distinctive in their morphology and development. In the retina at E6, they appear along the central portion of the optic streak. They have an ovoid soma and either unipolar or bipolar processes. From E6 to E13, these cells increase rapidly in number and can be gradually found in the peripheral region of the retina. At E13, the star shaped TFR positive glia is observed. These glial cells, in time, become the predominant population of TFR positive glia.


The distribution of neuropeptide Y (NPY) and dynorphin (DYN) in immature and mature male platyfish (Xiphophorus maculatus), a freshwater teleost, was studied using immunocytochemistry on Bouin’s-fixed, paraffin-embedded material. (ir)-NPY was found in perikarya and nerve tracts of the nucleus olfactoriotalis, telencephalon, ventral tegmentum and in the neophyposis and specific cells of the adenohypophysis. (ir)-DYN was found in nerve tracts in the olfactory bulb and in association with cells of the pars intermedia and caudal pars distalis of the pituitary gland. The association of NPY and DYN with brain structures and pituitary gland regions suggests that these neuropeptides play a role in these events.

541.6 THE DISTRIBUTION OF TAURINE-LIKE IMMUNOREACTIVITY IN THE AVIAN CENTRAL NERVOUS SYSTEM. D. A. N. M. Hasler, and J. D. Seevers. Department of Zoology, University of British Columbia, Vancouver, B.C. Canada V6T 1A9.

Taurine, or a taurine-containing dipeptide (γ-aminobutyric acid-glycyltaurine) has been implicated as a neuromodulator or inhibitory neurotransmitter in the CNS. We have used a monoclonal taurine antibody ("Taur," kindly provided by J. M. Maggio and B. Beitz) to examine the distribution of taurine-like immunoreactivity (TLI) in the brains and spinal cord of parafformaldehyde-perfused chicken (Gallus gallus) and horn (Anoa heroina) hatchlings. Many small and medium sized cells exhibit TLI in the brain. In the forebrain, especially in the striatum, hippocampus and parahippocampus, most of the immunoreactive cells appear to be neuronal. In the midbrain, brainstem and spinal cord the most intense TLI is seen in glia cells. In the cerebellum, a variety of cell types are immunoreactive, including glia, basket cells, and some purkinje cells. In general, the few large neurons which are immunoreactive (i.e. cerebellar pallidum cells and neurons in a few cranial nuclei) have only light positive staining. Moderate to intense TLI is also seen in the pineal. Throughout the brain (white and grey matter) many fibers exhibit light, medium or dark TLI. In the white matter (brain and spinal cord) many small intensely immunoreactive glial cell bodies are seen. At least some of these immunoreactive glial cells appear to be oligodendroglia. Thus, TLI is found in both neurons and glia and is widely distributed throughout the avian brain.
541.7
Rapid feasibility studies of new PET ligands: High resolution PET in small animals. M. Ingvar, L. Eriksson*, S. Sane-Blander*, G. Rogers*, H. Widler*, PET-Section, Karolinska Institute and Hospital, S-104 01 Stockholm, Sweden and Department of Chemistry, University of California Santa Barbara, CA.

The development of sufficient amounts of a radiotracer for use in PET is often a time-consuming process of optimizing radiolabelling yields and handling procedures. Sometimes the radiotracer is not chemically long, but rather a derivative with unknown in vivo biopharmaceutical properties. We have therefore developed a fast and relatively inexpensive method of generating new ligands in vivo. Small animal studies require relatively low amounts of radioactivity, can be performed without sterility and toxicology tests and may also serve as a preliminary basis for the design of future safety calculations since whole body scans are performed.

We have tested the procedure with an 18-F- analogue of Venlafaxine, a drug that specifically binds in vivo to synaptic vesicles of cholinergic neurons. For the procedure the rats were anaesthetised, ventilated and provided with arterial and venous catheters. The animals were placed in the PET scanner during the whole procedure and sequential scans were performed. After a bolus (i.e. injection of 30-100 µCi of the ligand repeated arterial samples were taken. The plasma radioactivity was determined to obtain the brain input function. Following image reconstruction of the scans standard time/activity plots were constructed. The camera (Scanditronix PC048-15B) has a resolution of 4.5 mm FWHM and a rat brain is approximately 16-25 pixels in the 128x128 image. The major advantage of this protocol is that very few experiments are necessary to reliably determine the kinetic properties of the blood brain barrier transport and the magnitude of whole brain binding to a receptor system. In vivo displacement of a ligand from a receptor can be performed with potentially toxic amounts of the drug. This is especially important since many receptors are characterized by a state-dependent binding that impairs a direct comparison of in vivo experiments with the in vitro situation.

541.8
PRESENCE OF IMIDAZOLOACID ACID RIBOSIDE, A METABOLITE OF IMIDAZOACID ACETIC ACID, IN HUMAN CEREBROSPINAL FLUID. G.D. Prell*, A.M. Morris*, E.Douyon*, and S. Hofkirk*. Department of Pharmacology, Mount Sinai School of Medicine, CUNY, New York, NY 10029 and Department of Neurology, Hackensack Medical Center, Hackensack, N.J. 07601.

Imidazol acetic acid (IAA), which we demonstrated in rat brain and in human CSF [J. Neurochem. 52; 1107, 1989], stimulates GABA-A receptors, produces analgesia, hypnotic, hypomimetic and other effects (see references). High concentrations of an hydroxylated conjugate(s) of IAA in brain (20 mmol/l) and CSF, whose levels exceed those of free IAA by up to 100-fold. We postulate that the conjugate(s) may have IAA-antagonist (Me-IAA) and/or IAA-antagonist (IAA-Rs).

IAA-Rs HCl, prepared for us by synthesis, was derivatized with triethyloxysilane (TMCS) and analyzed by gas chromatography (GC)-mass spectrometry (MS). IAA-Rs-TMCS was identified by chemical ionization (MIF 548) and electron impact ionization MS. We have extracted IAA-Rs from urine collected (<8 h) from rats multiply treated with IAA-HCl (p.o.) and D-glucose (p.o.). The TMS derivative(s) of IAA-Rs HCl prepared from urine and by synthesis showed identical GC and MS properties. Patients undergoing diagnostic or therapeutic procedures gave informed consent for collection of additional lumbar cerebrospinal fluid (CSF) for research purposes. In some patients, more than 30 ml of CSF was collected sequentially in 1-2 milliliter units. CSF aliquots of nl 4-5, 16-17 and 29-30, derivatized with TMCS, showed the presence of IAA-Rs. Derivatives of the latter showed GC-MS characteristics identical to those of IAA-Rs prepared from urine. This supports our previous postulate that IAA in brain may be ribosylated and suggests that IAA in CNS of humans may be metabolized by the only route of IAA metabolism known to occur in rodents within two distinct populations, one in the anterior, and the second in the postero-dorsal region of Me. Similar staining patterns were obtained with all ANT-antiseria. Animals not given colchicine (n=2) had only a few TH-immunoreactive cells in Me. Absence of DBH and PNMT-immunolabeling of Me suggested that these TH neurons are dopaminergic. Whether these DA neurons are involved in regulating sexual behavior will be examined in future studies (Supported by NS 52609 from NINDS).

541.9

Immunocytochemical studies with several antibodies raised against various suspected synaptic transmitters have been performed at the light microscope level to characterize the distributions of the neurotransmitters and axon terminals containing choline acetyltransferase (ChAT), Substance P, GABA, glutamate and aspartate in the lateral medial-supra-ventriculare complex (LM-SG) of the cat. Determination of the boundaries of LM-SG was aided by cytoarchitectonic criteria and by superimposition of adjacent sections treated for acetylcholinesterase (AChE).

The pattern of staining of varicosity-containing, ChAT-positive fibers corresponded well with the pattern of AChE staining seen throughout nearly all areas of LM-SG. Small neurons were heavily immunopositive for GABA. Aspartate and glutamate appeared to be present in moderate to low levels, and were found principally in larger neurons. Substance P staining was very weak and appeared to be located within a few small-diameter fibers that also possessed varicosities. These studies are continuing at the EM level to assess the likely nature of the transmitters of LM-SG afferents, particularly those from tectal and cortical sources. Supported by the Human Frontiers in Science Programme.

541.10

The medial nucleus of the amygdala (Me) integrates chemosensory and hormone signals influencing behaviors. The Syrian hamster (Mesocricetus auratus). Only in this species, to date, have dopamine (DA) neurons been described in Me (Vincent, J. Comp. Neurol., '88), although DA is considered to be important in rats. To extend Vincent's observations, we used colchicine to enhance tyrosine hydroxylase (TH)-immunostaining in Me. Nine adult males received intraventricular injections of 200 µg of colchicine and were perfused after 48 hours. Forty-six coronal brain sections were processed for peroxidase-antiperoxidase immunocytochemistry using 3 different TH-antibodies: one monoclonal (Inactar) or one of two different polyclonal antisera (Eugene Tech or East-Acres Biol.). Polyclonal antisera generated against DBH and PNMT (Eugene Tech) were also used to test for the presence of these other catecholamine synthetic enzymes. Unlike Vincent's observations, we found more than 100 TH-immunoreactive neurons within two distinct populations, one in the anterior, and the second in the postero-dorsal region of Me. Similar staining patterns were obtained with all TH-antiseria. Animals not given colchicine (n=2) had only a few TH-immunoreactive cells in Me. Absence of DBH and PNMT-immunolabeling of Me suggested that these TH neurons are dopaminergic. Whether these DA neurons are involved in regulating sexual behavior will be examined in future studies. (Supported by NS 52609 from NINDB).

541.11

Brain microdialysis techniques were used to examine effects of fenfluramine (10 mg/kg) and quipazine (3.0 mg/kg), both administered i.m., on serotonin (5-HT) release in the raphe and hippocampal areas of the pigeon. Dialysates were collected from the awake pigeon following earlier stereotactic implantations of a guide cannula targeted at the respective sites. Low serotonin and quipazine increased 5-HT concentrations in both brain regions, with peak effects occurring approximately 60 minutes following drug injection; effects of both drugs lasted for about two hours before returning to near control levels. These studies demonstrated that the microdialysis procedure can be used to study 5-HT neurotransystems in the awake, behaving pigeon. Supported by DA 02873.

541.12

The levels of monoamines (DA, NE and 5HT) and their metabolites (DOPAC, HVA, and 5HIAA) were measured in the brains of one week old chicks which were dissected into the following areas: thalamus (THL), habenula (HBH), neo-striatum/ectostriatum (NEO/ECTO), hyperstriatum (HS), parolfactory lobe/nucleus basalis area (LPO/RB), optic tectum (OT), cerebellum (CER) and spinal cord (SP). Tissue extracts were assayed using the Coulomb Electrode Array System of ESA Inc. (Bedford, MA.). In the NEO/ECTO, LPO/RB, THL and CER the levels of DA and DOPAC (mg/g tissue) were: 2 and 16, 92 and 227, 5 and 34, 108 and 99; NE & HVA were: 26 and 81; 30 and 52, 56 and 469, 526 and 41; and 5HT and 5HIAA were: 13 and 165, 306, 19 and 352, 206 and 306, respectively. The calcium-dependent release of 3H-DN from NEO/ECTO slices is inhibited by 5-ODD-tryptophan (1-1000 µM) and is increased by methiothen (1-1000 µM) suggesting modulation of release by 5HT autoreceptors. In addition the release of 3H-DN is modulated through DBH. The complete regional distribution of monoamines and release modulating presynaptic receptors will be presented. We conclude that in the chicken brain, as in mammals, presynaptic receptors modulate the release of monoamines. Supported by MH52922 to HLD and NS01740 to JAS.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
541.1


Melatonin binding sites are widely distributed throughout the chicken brain, predominantly in visual areas. The density and pharmacological characteristics of 2-[125I]-iodomelatonin binding sites were determined using quantitative autoradiography. Scatchard analysis of saturation data in representative areas of the chicken brain revealed saturable high affinity binding sites [K0 (pM): Max. (mol/mg protein) in the optic tectum (35: 11.4), nictitating (20: 10), supersaiuclastic nucleus (10: 6.9) and of rotundus (22: 11.2). Melatonin and 6-OH-melatonin were potent competitors of 2-[125I]-iodomelatonin binding [K0 (pM): OT: 62 and 117; NC: 8 and 63; vGSC: 19 and 47; and ROT: 57 and 68, respectively], while N-acetylsertotonin and luzindole show lower affinity [K0 (pM): OT: 93 and 633; NC: 107 and 188; vGSC: 292 and 491; and ROT: 56 and 149, respectively]. A complete regional distribution of density and binding affinities for 2-[125I]-iodomelatonin binding sites will be presented. We conclude that, high affinity 2-[125I]-iodomelatonin binding sites, with characteristics of ML-1 sites, are heterogeneously distributed throughout the chicken brain. Supported by NIH 29292 (MRO) and 5807710 (JAS).

541.2

NEUROENDOCRINE REGULATION: OTHER III

541.3


Recent studies performed in rat hippocampus indicate the presence of relatively high amounts of a protein which is indistinguishable from estrogen receptor (ER) by immunological criteria. To probe the estrogen receptor synthesis in this important limbic area, the presence of ER mRNA was tested by means of the polymerase chain reaction (PCR) technique. A PCR was therefore performed by oligo dT priming of mRNA extracted from the hippocampus of ovariectomized rats and a series of oligonucleotides were utilized in order to amplify selected portions of the coding sequence. This analysis proved the presence of ER mRNA in rat hippocampus. Sequencing of the amplified section of ER cDNA permitted to determine that the mRNA present in the hippocampus is identical to that one synthesized in uterus.

Further studies are presently being carried on in order to obtain a correct estimate of the concentration of ER in the rat hippocampus.

541.4

SHORT-TERM ELEVATION IN GLUCOCORTICOID LEVELS IS SUFFICIENT TO ELEVATE ADRENAL MEDULLARY PNMT LEVELS. P. Boka, K. Berto, and J. DiBiasio. Douglas Hospital Research Ctr., Dept. of Psychiatry & Pharmacology, McGill University, Montreal, Quebec, H4H 1R3.

A well known effect of glucocorticoids in the adrenal medulla is the regulation of pheochromocytamine-N-methyltransferase (PNMT) activity. PNMT catalyzes the conversion of noradrenaline to adrenaline in chromaffin cells of the adrenal medulla. Two days of glucocorticoid (GC) treatment has previously been shown to be required for significant elevation of PNMT levels in cultured bovine adrenal chromaffin cells. Since a short (1/2 hour) pulse of GCs is released in response to a single episode of acute stress, these studies examine the effects of treating cultured bovine adrenal medullary cells for a short time with high dose GCs, mimicking the physiological phenomenon, on PNMT activity 2 days later. Doses of 100 and 1000 uM of hydrocortisone (HC) were used in the treatments, and in all studies no differences were found between the two doses. Similar to previous results by others, continuous 2 day HC treatment increased PNMT levels to 247% of control. Acute HC treatments of 30 minutes or 2 hours elevated PNMT levels assayed 48 hours later to 174% & 199% of control, respectively. The increase in PNMT levels that resulted from a 2 hr pulse of HC persisted for 72 hrs following treatment. Preliminary data suggest that a 2 hr pulse of HC (100 uM) begins to elevate PNMT levels within 12 hours. These results suggest that short-term elevation in GC levels, as seen physiologically after acute stress, is sufficient to elevate adrenal PNMT levels. Supported by MRC of Canada and FRSQ.
543.5 EVENING MELATONIN INJECTIONS INHIBIT TURBOFUNDIFORMULAR DOPAMINE SYNTHESIS IN OVARECTOMIZED HAMSTERS. N.A. Alexiou and J. Vriend, Dept. of Anatomy, Univ. of Washington, Seattle, WA 98195.

In the present study the effects of melatonin administration on the accumulation of serotonin (5HT) and dopamine (DA) in the pargyline activated area were studied in tissue punches of median eminence and of caudate n. of intact and ovarectomized Syrian hamsters. Concentrations of 5HT and DA were determined by HPLC with electrochemical detection. In median eminence, but not in striatum, the accumulation of DA was reduced to 22% of controls in intact hamsters (p < .05) and to 9% of controls in ovarectomized hamsters (p < .01) by melatonin injections. Ovarectomy resulted in a significant increase in DA accumulation after pargyline in saline-injected, but not in melatonin-injected hamsters. No significant effects on the accumulation of 5HT in median eminence or in striatum could be detected. These data show that evening melatonin injections inhibit daytime DA synthesis. The results suggest that melatonin may influence the activity of tyrosine hydroxylase in median eminence directly, or indirectly via serotonergic neurons which synapse on tyrosine hydroxylase containing neurons in the median eminence. (Supported by NIMH).

543.7 EFFECTS OF TESTOSTERONE PROPIONATE ON THE DENSITIES OF 5-HT1A AND 5-HT1B RECEPTORS IN THE BRAINS OF CASTRATED MALE RATS. S.D. Mendelson and B.S. McDaniel, Laboratory of Neuroendocrinology, Rockefeller University, New York, NY 10021.

Gender hormone levels modulate the density of 5-HT1 receptors in the male rat brain. However, it has not been known what effects the hormones might have on the subtypes of 5-HT1 receptors. Effects of activation of 5-HT1A and 5-HT1B receptors on testosteron-dependent sexual behavior have suggested that these receptors may be gender hormone-dependent. Accordingly, male rats were castrated under anesthesia and administered either testosterone propionate (TP, 0.2 mg) or vehicle to the oil vehicle in 14-daily injections. Labeling of 5-HT1A and 5-HT1B receptors for autoradiographic analyses were accomplished by incubation of brain sections in tri- buffer solutions of 1-15 nM [3H]-hydroxy-(dipropylamino)etramine or 50 pM[125I]cyamopindolol plus isoproteneol, respectively. In each case, unlabelled serotonin was used to determine non-specific binding. TP produced significant increases in the density of 5-HT1A receptors in the mediolateral preoptic nucleus (MPO), but had no effects on these receptors in areas that included the ventromedial hypothalamus, septum and cortex. TP had no effects on 5-HT1B receptors in any area measured, indicating that testosterone can have differential effects on the subtypes of 5-HT1 receptors. The present data suggest that 5-HT1A receptors in the MPO may mediate facilitation of male sexual behavior in the rat.

543.8 GONADAL SEX HORMONE DIFFERENCES IN THE BRAINS OF CASTRATED MALE RATS AND THEIR CONSEQUENCES.  T. Turner, B.W. Ross and R.F. McKeehan, Department of Psychiatry, Emory University School of Medicine, Atlanta, Georgia 30322 and the Georgia Mental Health Institute, Atlanta, Georgia 30330.

In long- and short-term castrates, castrated male macaques, testosteron administration does not suppress plasma gonadotropin levels or always fully reinitiate coital behavior. To examine the proposition that long-term castration alters the uptake of androgens by the brain, 4 adult male rhesus monkeys were castrated an average of 8.25 years (range 6-10). Sections were cut in a cryostat at 4 μm, thaw-mounted on emulsion-coated slides and exposed for 20-40 weeks. A total of 8,800 neurons in 11 brain regions were examined using a computerized grain-counting system (Bioquant MG IV, RAM Biometrics), and labeled neurons were identified using a rigorous criterion based on the Poisson distribution. In both short- and long-term castrates, there were significant regional differences in the percentages of labeled neurons (p<0.001). Highest percentages (50%-85%) occurred in the ventromedial and anterior hypothalamic nuclei, cortical and basal accessory amygdaloid nuclei, intercalated mammillary and preglomerular nuclei, bed nucleus of the stria terminals (BST), and medial preoptic area. Lower percentages (20-50%) occurred in the lateral septum, arcuate nucleus, and medial amygdala. However, there were no differences in the percentages of labeled neurons between short- and long-term castrates with the sole exception of the BST. Data derived from computerized grain-counting correlated highly (r=0.8, p<0.0001) with those derived from manual counting methods (USPHS grant MH 5956).


Gonadal hormone-dependent and -independent sex differences exist in GOCR binding sites in rat brain (Turner & Weaver, 1985). Because neuronal and lymphoid GOCR may play different roles in various tissues, we examined sex differences and hormone sensitivity in thymus and spleen weight and thymocyte and splenocyte GOCR sites/cell in 60-day-old gonadectomized (GDX, n=10) and gonadally intact male and female Sprague Dawley rats. There were no sex differences in spleen or thymus weight for either intact or GDX rats. GXR increased thymocytes in males (p<0.004) and females (p<0.01), but had no effect on spleen weight. No sex differences occurred in GOCR sites/cell (using 64 nM [3H]trilostene, a GOCR ligand) in intact male or female GDX rats. GXR increased GOCR number in male thymus (2.4-fold, p<0.01), but not in females. Splenocyte GOCR number was not affected by GXR. Our data indicate that thymus, but not spleen, weight and glucocorticoid receptor number are depressed by gonadal hormones in the rat, suggesting that GCHR may provide a possible mechanism for gonadal hormone modulation of the effects of stress on immune function. (NIH HD07228 & HD0182, Bethising, MD & VA Medical Res.)

543.10 AUTORADIOGRAPHIC LABELING OF NEURONS IN THE BRAINS OF SHORT AND LONG-TERM CASTRATED ADULT MALE RHEUS MONKEYS AFTER ADMINISTERING [3H]TETRAHYDROSTERONE. A.N. Chang, B.W. Ross and R.F. McKeehan, Department of Psychiatry, Emory University School of Medicine, Atlanta, Georgia 30322 and the Georgia Mental Health Institute, Atlanta, Georgia 30330.

In long- and short-term castrates, castrated male macaques, testosteron administration does not suppress plasma gonadotropin levels or always fully reinitiate coital behavior. To examine the proposition that long-term castration alters the uptake of androgens by the brain, 4 adult male rhesus monkeys were castrated an average of 8.25 years (range 6-10). Sections were cut in a cryostat at 4 μm, thaw-mounted on emulsion-coated slides and exposed for 20-40 weeks. A total of 8,800 neurons in 11 brain regions were examined using a computerized grain-counting system (Bioquant MG IV, RAM Biometrics), and labeled neurons were identified using a rigorous criterion based on the Poisson distribution. In both short- and long-term castrates, there were significant regional differences in the percentages of labeled neurons (p<0.001). Highest percentages (50%-85%) occurred in the ventromedial and anterior hypothalamic nuclei, cortical and basal accessory amygdaloid nuclei, intercalated mammillary and preglomerular nuclei, bed nucleus of the stria terminals (BST), and medial preoptic area. Lower percentages (20-50%) occurred in the lateral septum, arcuate nucleus, and medial amygdala. However, there were no differences in the percentages of labeled neurons between short- and long-term castrates with the sole exception of the BST. Data derived from computerized grain-counting correlated highly (r=0.8, p<0.0001) with those derived from manual counting methods (USPHS grant MH 5956).
543.11 NEUROENDOCRINE BLOCKS ERECTIC SYMPATHETIC RESPONSES OR RAT SUPRAOPTIC NUCLEUS (SON) NEURONS TO LATERAL OLFACTORY TRACT (LOT) STIMULATION. K. G. Smithson & G. L. Hatton. Neurosciences Program & Physiology Dept., College of Osteopathic Medicine, Michigan State University, East Lansing, MI 48824.

The innervation of the SON by the LOT (Hatton, G. L. & Yang, Y. . Neuroendocrine Cytology 4, 271, 1989) and sensitivity (Smithson, K. G. et al. Anat. Rec. 228:91A, 1988) olfactory bulbs project to the SON. Intracellular electrophysiological analysis of this connection in brain slices revealed predominantly short-latency excitatory responses to electrical stimulation of the LOT (Hatton, G. L. & Yang, Y. . Neuroendocrine Cytology 4, 271, 1989). Since such evidence suggests that neural activity in the olfactory bulbs employ excitatory amino acids (EAA) as neurotransmitters, we investigated the possibility that short-latency responses observed in SON neurons were mediated through an EAA receptor. Using an excitant preparation that contained virtually the complete olfactory projection from the rostral end of the LOT to the SON, we examined the effects of bath application of 1 mM kynurenic acid (KY) on the evoked responses in SON neurons from LOT stimulation. Intracellular recordings were obtained from 66 SON neurons, of these, 50 neurons responded to electrical stimulation of the LOT. The responses observed were all excitatory and had variable latencies. In 15 neurons in which long-term stable impalements were maintained, KYN blocked LOT-evoked responses. In all cases, action potentials could be evoked during the KYN blockade by intracellular current injection, or when previously possible, antidromically by neural stimulation. These results confirm previous reports of the excitatory nature of this connection. An additional finding in the present study is that these excitatory responses are blocked by KYN's specific antagonist of EAA receptors, a result supporting the notion that this cell neurotransmission is mediated, at least in part, via EAA's. Supported by NS 16942 and by a fellowship from a Medical Scientist Training Program to KJS.


The distributions of aromatase (ARO) and estrogen receptors (ER) were studied in the brain of intact male Japanese quail (Coturnix c. japonica) by double label immunocytochemistry using a polyclonal antibody against human placental ARO (J. Biochem. 103: 106, 1988) and Abbot's monoclonal antibody H2222 against human ER. Absent aromatase-immunoreactive cells (ARO-ir) were found in the medial preoptic nucleus (POA), in the septal region, and in a large cell cluster extending from the doro-lateral aspect of the ventromedial nucleus to the tuberal region of the hypothalamus. Estrogen receptor-immunoreactive cells (ER-ir) were also found in these brain areas, but their distribution was much broader and included larger parts of the preoptic, septal, and tuberal regions. Only a limited proportion of cells were double-labeled (3%-30% in PM, <5% in the septum but more than 90% in the tuber). At the EM level, ARO-ir was limited to certain neurons and filled the entire perikarya, including presynaptic boutons. There were ARO-ir positive boutons forming synapses with ARO-ir neurons (Abst.669, The Endocrine Society, 1990). CONCLUSIONS: The present study shows that ERs are more broadly distributed and only present in some of the ARO-ir cells. These findings indicate that in addition to the usual ER-mediated actions, locally formed estrogen may have non-ER mediated actions involving the synaptic level and elsewhere in brain neurons. Supported by NIH HD22064, NRS and EEC (SCI-030-CTT) to JB, NS26068 and NIH HD23835 to CL and NIH HD13587 to FN.


Following injections of phasostigmus vulgaris leucocochyatus, PHA-L, and rhodamine isothiocyanate into the optic tectum (ION), the laminar distribution and morphology of ION afferent fibers to the retina (centrifugals) were studied in transverse and horizontal sections.

In transverse sections through the retina, while both markers revealed a heavy band of terminals confined to lamina b of the inner plexiform layer (IPL), and fibers penetrating into the inner nuclear layer, PHA-L also showed sparse labelling in lamina Sb. Immunocytochemical studies have indicated that 5-HT is also located in lamina b of Sb and the IPL. Horizontal sections through the retina showed that fibers entering the optic nerve head, which is bicortical, ranged in diameter from 0.1 to 1.0. Three types of terminal arborizations were observed: Type 1: thin fibers expanding over a large area of the retina, having as many as 70 terminal arborizations, Type 2: thin axons, terminating over a confined surface area, appearing at 80 terminal endings, and Type 3: thick fibers, having a restricted terminal field with as many as 60 terminal boutons. While there was little variation in the number of terminations for the three categories of fibers, the terminal surface area occupied by type 1 axons was approximately 7 times greater than the other types of axonal terminal endings.

A large number of amacrine and displaced ganglion cells were influenced by single centrifugal fibers, either over a widely spaced or confined surface area of the retina. Moreover, centrifugal fibers could modulate 5-HT positive amacrine cells, suggesting that the ION modulates various subpopulations of morphologically and biochemically distinct amacrine cells in the retina.

Supported by a Ford Foundation Post-Doctoral Fellowship to W.W. and NEI grant EY05876-02 to W.W. and EY08990-05 to HJK.


Intracellular recordings were made from arcuate (ARC) neurons with biocytin-filled electrodes in slices prepared from ovariectomized guinea pigs treated with estradiol or oil. Fifty-seven neurons were identified and immunoreacted for $\beta$-endorphin (BEND). Fourteen of these cells were immunopositive for BEND. BEND neurons had membrane characteristics (i.e. RMP: -56 ± 1 mV, Rm: 380 ± 67 mΩ: 16.5 ± 4 ms) similar to immunonegative neurons and often fired spontaneously (6.1 ± 20 Hz). BEND neurons exhibited an instantaneous as well as time-dependent rectification. A population of BEND neurons (N = 6) exhibited an I3. The µ-opioid agonist Tyr-D-Ala-Gly-NoPro-Gly-ol (BAXS) induced membrane hyperpolarization (12 ± 2 mV) and decreased the Rm (38 ± 4 ms) of the BEND neurons. This population was similar in non-BEND neurons. In preliminary experiments, the ERP for the effects of DAMO (-0.15 mV) was similar in cells recorded from oil- and estradiol-treated animals. The pA2 values for naloxone antagonism of the effects of DAMO in both groups were in the range reported for other tissues. Experiments are in progress to determine if estrogen causes any shift in the pA2 value. Thus, µ-receptors may be autoreceptors on ARC BEND neurons, and this "ultra-short loop" feedback mechanism may be modulated by estrogens. (PHS DA 05158, HD 00718).
453.3
THE MEDIAL BASAL OPTIC TRACT IS COMPRISED OF AXONS ORIGINATING FROM GANGLION CELLS IN THE CENTRAL RETINA OF PROGS. Z. LIFH AND K. V. FLIT.

Neuroscience and Behavior Program, University of Pittsburgh, Pittsburgh, PA 15260

The nucleus of the basal optic root (NBOR) of anuran amphibians is innervated by the basal optic root (BOR) which includes the medial (BOM) and lateral (BOL) fascicles. BORs innervates the mediadorsal portion of NBOR and 98.5% of its axons are unmyelinated (mean = .27 \pm .23 mm). In contrast, BOL innervates the ventral-lateral and central portions of NBOR and contains many more myelinated axons. Unmyelinated axons are of larger caliber (mean = .42 \pm .23 mm) than in BOR (Flit, et al., 1988).

Large, efferent ganglionic neurones occur in the mediodorsal portion of NBOR and project exclusively to the dorsal or ventral portion of the pretectal nucleus lentiformis mesencephali (LdM). The dorsal core of NBOR receives optic afferents from the central portion of the dorsal retinas and contains large neurones which project to the optic tectum (Montgomery, et al., 1985).

Localized SEP injections of mediodorsal NBOR retrogradely labelled axons only in BOR and small ganglion cells in the central one-third of the contralateral retina. Thus, the projection from NBOR to LdM may also convey information from the central retina, with specific effects upon large neurones of NBM and its function in ocuomotor reflexes and optokinetic nystagmus (Supported by NSF grant: BNS 8819970).

453.4
GAD-LIKE IMMUNOREACTIVITY IN CENTRAL VISUAL AREAS OF RANA PIPIENS. C. J. TULGAR, K. V. FLIT, AND G. J. DE VRIES.

Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003

Immunohistochemistry was used to demonstrate the presence of glutamic acid decarboxylase (GAD), the synthesizing enzyme for gamma-aminobutyric acid (GABA), in primary retinal terminal fields and thalamic nuclear groups that project to primary retinal nuclei in frogs (Rana pipiens).

Preliminary observations revealed GAD-like immunoreactivity in all primary visual nuclei. Dense GAD-like immunoreactive (GAD-LIR) puncta were observed in the posterior thalamic nucleus, uncinatus, and nucleus lentiformis mesencephali (LM). In addition, GAD-LIR perikarya were observed positioned along the lateral and ventral margins of LM. Present evidence suggests the presence of GAD-LIR terminals in all lamina of the optic tectum, with GAD-LIR perikarya appearing most numerous in the 6th and 9th laminae. The lateral margins of the basal optic root nucleus contained scarce populations of GAD-LIR perikarya which surrounded the GAD-LIR puncta within the nucleus itself. Both the corpus geniculatum and nucleus Belonii contained GAD-LIR fibers and puncta. Magnocellular neurons in the nucleus profundus also showed dense GAD-LIR perikarya and dendritic morphology. In the thalamus, the ventrolateral area, posterolateral nucleus, and posteromedial nucleus (cell groups in contact with retinal afferents) contained both GAD-LIR perikarya and puncta. Comparisons between GAD- and GABA-like immunoreactivities in these areas will also be presented. (Supported by NSF Grant BNS 5-25576 to KVF.)

453.5
EVIDENCE FOR DIRECTION-SENSITIVE (DS) RETINAL INPUT TO THE TURTLE'S BASAL OPTIC NUCLEUS (BON). A. E. ROSENBERG AND M. ARIS.

Dept. of Behavioral Neuroscience, University of Pittsburgh, PA 15217

Turtle BON cells respond best to global image motion in a preferred direction (Rosenberg & Aris, 1990), consistent with their purported role in encoding retinal images. The DS character of BON cells is thought to be signaled by DS retinal ganglion cells (RGC) via the direct contralateral retinal input, based on evidence that RGCs are (1) DS in the absence of the telenchelar bats, but (2) not DS during retinal bicuculline application (Scherger et al., 1989).

To test this idea, DS RGCs project directly to the BON, RGCs were characterized during single unit recordings and then were tested for antidromic activation by electrically stimulating the BON. The preparation was an ad lib turtle brain, with the eyes, telenchelar and telenchelar removed. RGCs were characterized based on their response to steps of diffuse illumination and moving whole-field checkerboard patterns. Current passed through a bipolar electrode was used to stimulate the BON.

Of 34 RGCs recorded, 5 could be activated antidromically using current < 200 \mu A. Four of these were DS. Antidromic spikes had latencies ranging from 1.6 to 3.8 ms, and could follow a stimulus train exceeding 100 Hz. These latencies were slower than those of antidromic spikes (2.5 - 7 ms, n = 15) recorded from BON cells, elicited by contralateral optic nerve stimulation. Orthodromic spikes recorded in the BON failed to be evoked by consecutive current pulses delivered to the optic nerve at stimulus rates > 30 Hz. Based on these measurements, the direct retinal input to the BON is moderate to rapidly conducting, as suggested by Woodbury and Ulinski (1986). This antidromic activation of DS RGCs provides direct evidence for retinal processing providing direction-sensitivity to BON neurons. (Supported by EY05978)

453.7
DISTINGUISHING ROTATION FROM TRANSLATION: NEURONS IN PIGEON VESTIBULOCECUBELUM SPECIFY DIFFERENT PATTERNS OF WHOLEFIELD MOTION. D. R. WYTHE AND B. L. BEST.

Dept. of Psychology, Queens University, Kingston, Ontario, Canada, K7L 3N6.

Several studies have demonstrated that the Accessory Optic System (AOS) is involved in the analysis of wholefield visual motion which results from self-motion of an animal. Earlier studies have found that neurons in the pigeon AOS respond best to wholefield visual stimuli moving in a particular direction (Wright and Petruska, 1983), which are aeronautical reference frames. For example, the potential for binocular interactions. It was the purpose of this study to investigate the responses of neurons in the pigeon VGC to wholefield visual motion. Pigeons were anaesthetised with urethane and extracellular recordings were made with glass covered tungsten microelectrodes. Complex spikes were found in the lateral dorsal and ventral portions of the pigeon VGC. Orthodromic potentials isolated outside the Purkinje layer responded to wholefield motion. Some functional types were found: neurons preferring upward motion in one eye and downward motion in the other eye; neurons preferring downward motion in both eyes, which would result from upward translation; and neurons preferring backward and forward motion in the contralateral eye and lateral eye, respectively, which would result from a horizontal head or body rotation. Binocular wholefield motion of the preferred direction in both eyes always resulted in a facilitation of the response relative to the dominant eye. A few presumed granule cells responded to monococular wholefield motion. We conclude that neurons in the VGC integrate wholefield information from the two eyes so that self-produced translation can be distinguished from rotation.

453.8
FUNCTIONAL ORGANIZATIONS IN THE NUCLEUS ROTUNDUS OF PIGEON: Y. C. WANG AND B. L. BEST.

Departments of Physiology and Psychology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Anatomical studies have shown that different retinal zones receive input from different retinal laminae and in turn project to different areas of the midbrain. Our previous results indicated that there were at least 6 different types of stimulus specific cells in the nucleus rotundus (RO), and in this study on Ketamine/Rompun anaesthetized pigeons, we report correlations between these functional classes and their location within Rt. We have found that most laminae units were located in ventral zones of the nucleus. These units were very large receptive fields (100-140 degrees on average) and either systematically increased or decreased their responses to increases in luminance of the whole visual field. In contrast, most lamina units were found in the dorsal-medial part of the Rt. These units respond to Z-axis movement, that is expanding or contracting stimulation patterns, and exhibited neither X-Y directional selectivity nor "on-off" responses. Oscillation sensitive units, both of the excitatory and inhibitory type, were mostly located in posterior Rt zones which receive input from deep tectum. We have also found that although some lamina and oscillation units decrease their response to changing wavelengths, most of them still give reliable responses to equiluminous chromatic stimulation movements. Supported by NSERC grant A0353 to BJJ.
543.9 ULTRASTRUCTURAL CHARACTERISTICS OF THE MEDIAL PRETENTAL NUCLEUS IN THE CAT. B. Pismans, A.D. Pearse, and B. Hutchins. Baylor College of Dentistry, Dallas, TX. 75246

The present study was undertaken to analyze the ultrastuctural characteristics of the medial pretectal nucleus, located at the mesencephalic junction. According to the predominant usage of the term, the following nuclei were identified within the nucleus, RLD, RSP, LLP, and PD. All terminals were found to be extraneuronal. The predominant terminal was of the presynaptic dendrite type, the PD, which were primarily found to contact medium sized dendrites. RLD terminals, which have been identified as different terminals to the pretectal complex (e. g. visual cortex, Hutchins and Weber, Anat. Rec. 205 (83); Neurosci. Abst. 19 (89); superior colliculus, Lieberman, et al. Neurosci. Lett. 56 (85)), were observed to contact small to medium size profiles. Fewer profiles were of the presumptive retinal terminal type, the RLP, which were found to have been identified as different terminals to the pretectal complex (e. g. visual cortex, Hutchins and Weber, 1983). Thus, one would expect to find the terminal of the PD is a neuron. This hypothesis was tested by the use of DAB electron microscopy. In this tissue, a few small terminals were labelled with HRP. Thus, this study provides preliminary data demonstrating direct retinal input to the medial pretectal nucleus in the cat.

Supported by NIH grant EY06977

543.10 THE VISUAL ORGANIZATION OF THE POSTERIOR PRETENTAL NUCLEUS IN THE CAT. B. Hutchins and B. Pismans, Baylor College of Dentistry, Dallas, TX. 75246

The pretectal complex is located at the mesencephalic junction and is composed of five separate nuclei. After tissue is processed for either an HRP or a Ni-proline injection into one eye of an adult cat, the direct retinal projections to the pretectal complex were identified. One little studied pretectal nucleus, the NEP, receives a patchy, bilateral input. These nuclei appear to follow the dorsal posterior border and extend the entire medial to lateral extent of the nucleus. This leaves the majority of NEP without identifiable projections. To test whether the remaining portions of NEP are visually related, the nucleus was sampled electrophysiologically in paralyzed chloral anesthetized cats. Data collected thus far, indicated the full extent of NEP is visually responsive. The remaining portions of the vertical meridian were identified more anterior and the lower visual field was identified later. In addition, approximately one half of the extracellular tissue sample responded to auditory stimuli. Thus, these preliminary data indicate a complex role for NEP in integrating multisensory information.

Supported by NIH grant EY06977

CONTROL OF POSTURE AND MOVEMENT: CLINICAL STUDIES

544.1 ARE THE ABNORMAL MOVEMENTS OF TARDIVE DYSKINESIA COMPLETELY BARKENQ. JR Lory, T. Ingew, and NF Calligliotta. Motor Function Laboratory, VA Medical Center, V-116A, San Diego CA 92161

One of the clinical hallmarks of choreoathetoid disorders is their spatial irregularity. In the case of tardive dyskinesia, however, questions have been raised as to how truly random the movements are. In an effort to address this question, we attached a biplanar accelerometer to the tip of the thumb of the more dyskinetic hand in patients with TD of varying degrees of severity and compared the data to those of healthy volunteers. Data were analyzed by plotting the acceleration values associated with the abnormal movements in cartesian coordinates. We then quantified the proportion of activity associated with a particular acceleration vector, yielding an r value, which is a measure of the spatial irregularity. Results showed that greater r values were associated with more severe TD, indicating that with increasing severity of the dyskinesia, the movements were more regular. Therefore, the movements of patients with milder TD are not completely random, but do show spatial regularity. These results will be contrasted with similar measurements made in patients with Huntington’s disease, the prototypic choreoathetoid disorder.

Supported by NIH grant AG03991

544.2 DIMENSIONALITY OF POSTURAL STEADINESS. J.R. Myklebust, T.E. Prior, IM Myklebust, DG Lange, Marquette University, College of Engineering, Laboratory of Sensory-Motor Performance, VA Medical Center Medical College of Wisconsin, 8100 W. Wisconsin Ave., Milwaukee, WI 53295 and VA Hospitals & Insf NINDS-NINDB, Bethesda, MD. Deformities of the center of pressure (CP) during quiet standing have been characterized for healthy adults. These measurements have been used to assess neurological disorders of vestibular and cerebellar systems, and lesions of spinal and extrapyramidal tracts. In previous studies1 the fractal dimension of the planar curve was used to analyze balance data. The algorithm was developed for segmental curves and may be a simple, practical method to simplify the use of chaos theory in the present study. The fractal dimension, computed using the “coastline method”, and a box counting method were compared to the previous algorithm. The slope of the fractality spectrum was a power law function. For comparison, the time series was embedded in higher dimensions and the correlation dimension computed2. Data was used from 5 normal young adults (age<40), 5 healthy aging subjects (age 55-65), and 5 elderly patients with Alzheimer’s disease with clinical impairments of gait and balance. Standing balance trials were sampled at 10Hz for 1 minute with eyes open and 1 minute with eyes closed. The CP was analyzed for each trial. The fractal dimension obtained by the coastline and box counting methods did not differ significantly from the value computed using the previous algorithm. The mean value tested with eyes open was 1.97 (sd=0.25) for young subjects, 1.73 (sd=0.06) for healthy aging subjects and 1.58 (sd=0.09) for Alzheimer’s patients. With eyes closed, the normal subjects did not change; the Alzheimer’s group improved to 1.79 (sd=0.05). The slope of the power spectrum was 2.5 for young adults, 3.0 for healthy aging subjects and 3.2 for Alzheimer’s patients. The correlation dimension calculated from the embedded time series was lower than the fractal dimension.

1. Myklebust JR, Myklebust IM: Fractals in Kinesiology, Soc Neurosci Abs 13, 1043, 2.4g: 404, 1980. 2. Mayer Kessler G: Dimensions and Endpoints in Chaotic Systems. Springer-Verlag, NY, 1985. This work has been supported by funds from VA Rehabilitation R&D.

544.3 EVIDENCE FOR SEPARABLE FACTORS OF ALZHEIMER’S AND PARKINSON’S DISEASE ON MOVEMENT PREPARATION AND EXECUTION PROCESSES. JC Amchine, Dept. of Psychology, Univ. of New Mexico, Albuquerque, NM 87131, and IC Morris, Dept. of Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

A study was conducted to determine the effects of Alzheimer’s Disease (AD) and Parkinson’s Disease (PD) on cognitive/motor processes underlying motor plan preparation and execution. Using 21 AD: Absent or Present X 2 (PD: Absent or Present: [remediated] design, four subject groups: 10 control subjects: mean age = 71.1 yrs, 6 AD subjects: mean age = 73.1 yrs, very mild dementia, 30 yrs, no dementia, and 5 AD/PD subjects (mean age = 69 yrs, very mild dementia) were compared to right-handed healthy volunteers with upper extremity movement for reaction time (RT) and movement time (MT). A paradigm was used where 79% of the trials had valid precise stimuli (i.e., identical to the target stimuli) but the remaining 21% were equal stimuli (i.e., different from the target stimuli), thus necessitating restructuring of the prepared response. Subjects responded to one of four target stimuli by pressing a corresponding button. Group differences concern overall RT and MT: AD, but not PD, increased RT [F(1,28) = 4.31; p < 0.05 and F = 1, respectively]. However, AD and PD inhibited additive effects in increasing MT [F(1,28) = 6.08, p < 0.05 and F(2,20) = 15.7, p < .001, respectively]. Results indicate that AD (at a very mild level of dementia) and PD independently influence cognitive/motor task performance: AD, but not PD, slows motor plan preparation (RT); however, AD and PD each slow motor plan execution (MT).

Supported by NIH Grant AG10391

544.4 MOTOR LEARNING IN PARKINSON’S DISEASE: SCHEMA FORMATION, ADAPTATION TO ALTERED GAIN, AND LIMB KINEMATICS. C.J. Worringham, C.L. Cross and A.L. Smiley-Open. Department of Kinesiology, The University of Michigan, Ann Arbor, MI 48109.

Motor learning in Parkinson’s disease (PD) studied in a task requiring the learning of an arbitrary relationship between the length of visually cued bars and the amplitude of linear, horizontal arm movements. Four targets were initially learned with error feedback. Learning and extrapolation were assessed using no-feedback trials to, respectively, the original targets and targets beyond the range of those previously practiced. A final phase required adaptation to an altered gain. PDs were capable of learning the initial task, and, along with controls, exhibited a range effect (shorter wrong targets and undershoot, respectively). On extrapolation, the slope of the function relating actual to required movement amplitude fell sharply for PDs, with larger undershoots of longer targets. PDs showed substantial adaptation to a new gain within 164 trials. PD and kinematic data show that the formation and use of higher level “schema” representations in motor tasks is not lost in PD but in some subjects is degraded. Supported by NINDS grant 1R29 NS27761-01
SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

544.5 SENSORIMOTOR DISINHIBITION IN PARKINSONISM MP Caliguri, SC Heidel and JE Lohr, Motor Function Lab, VA Medical Center, San Diego, CA 92161 The inability to execute simultaneous movements is well recognized in Parkinson's disease (PD). Investigators have proposed a number of hypotheses to explain this inability, including deficits in attention, motor programming, or both. In the present study we investigated the role of sensorimotor disinhibition as a mechanism underlying the inability of PD patients to execute simultaneous motor acts. The patients were examined after a 10-h drug-free interval. The motor examination required that the subject perform a task of isometric-index flexion force for 20 seconds under two conditions: (a) with the non-test hand at rest and (b) with the non-test hand engaged in a RT test in which ballistic force pulses served as the response to auditory stimuli. Analyses were made of the degree and pattern of force instability. Results indicated that the PD patients exhibited significantly greater isometric instability than controls. Force instability increased in both control and PD groups during the RT task; however, this increase was significantly greater for the PD than the HC groups. Further analyses revealed that the isometric force waveform for the PD patients, but not the controls, varied systematically with the RT pulses of the contralateral hand. This pattern of coherence for the two hands may be explained by sensorimotor disinhibition. In PD, the motor commands for executing ballistic muscle force (RT) may be uninhibited and pass through the system programmed for isometric control.

544.6 ABNORMALITIES IN GAIT INITIATION IN PATIENTS WITH PARKINSON'S DISEASE. K. Takahashi*, E.G. Lee, and K.J. Becker. Dept. of Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada T2N 4N1. Patients with Parkinson's disease (PD) often show difficulty in gait initiation. To investigate this further, we studied the timing of the onset of trunk and leg during the initiation of walking from the standing position. Shoulder, hip, knee and foot movements were recorded with a Vistamark positron emission analysis system and recorded with surface electrodes from the lower leg muscles during the 1st step while patients and controls began to walk in response to an auditory tone. Proceeding toe off of the swing leg, prominent EMG activity occurred in the pretilibial muscles bilaterally, the knee of the stance leg began to move forward and thus forward movement of the shoulder and hip occurred in both patients and controls. Forward movement of the shoulder and hip followed the initial forward displacement of the stance knee with a shorter time interval in patients than controls. Patients with PD showed a greater delay in gait initiation in response to the auditory stimuli and a longer time interval between both the ankle and leg movements. EMG onset and toe off of the swing leg than controls.

In gait initiation, patients with PD show not only delayed initiation of movement, but also a disordered pattern of leg and trunk movements.

544.7 DURATION OF POSTURAL RESPONSES IN CLUMSY CHILDREN: ANOTHER LOOK AT TIMING CONTROL. H. Williams, S. Jay*, N. Woolliscroft. Motor Control Laboratory, U of Oregon, Eugene, Oregon 97403. Skilled movement requires appropriate timing of muscle activity. Children with developmental apraxia are known clinically to have difficulty performing a wide variety of motor tasks 1 have been shown to have disturbances in both sequencing of postural responses and in timing of rhythmic, repetitive movements of distal body parts. The purpose of the study was to further examine timing control in clumsy children by studying duration of muscle activity as a function of the presence or absence of vision, with modified or normal vestibular input. 3-way repeated measures MANOVA's were used to analyze mean & variability of duration of EMG activity in 6 muscle groups. Duration of muscle activity was longer in clumsy children & was significantly longer with vision than without. With modified vestibular input, both clumsy and normal children showed increased duration & variability of postural responses. These data suggest that clumsy children may have widespread problems with timing of muscle responses integral to movement control.

544.8 EMG CHANGES IN GAIT FOLLOWING TENDO-ACHILLES LENGTHENING IN CEREBRAL PALSY CHILDREN. B.R. Emery1, C.S. Chambers2 and N.H. Scarborough1. 1Human Performance and Health Sciences Unit, Rice University, Houston, TX 77251, 2Gait Analysis Laboratory, Shriners' Hospitals for Crippled Children, Houston Unit, Houston, TX 77030. Due to spasticity, children with cerebral palsy typically demonstrate an equinus gait from overactivity of the triceps surae muscles. Surgical correction for the equinus gait pattern is done either by lengthening the Achilles tendon or recession of the gastrocnemius. The purpose of this study was to determine the effect of recession on muscle activity & if one surgical method could be considered more effective for reducing muscle activity during gait. Twenty-three hemiplegic or diplegic children participated both pre- and post-operatively in a gait analysis laboratory. EMG was collected using surface electrodes applied over the triceps surae muscle of the involved limb or limbs. Percent time of muscle activity during each step cycle was calculated for each subject. Analysis of variance revealed significant reductions of muscle activity during the gait cycle between pre-operative (64.0%) and post-operative (50.4%) measures, F(2,15) = 4.75, p < .001. No significant difference in EMG for percent of gait cycle between the two surgical methods was observed. It was concluded that either surgical method of Achilles lengthening was effective in reducing the spastic activity in triceps surae muscles of cerebral palsy children. These structural changes were apparently effective in changing either the level of spastic response or the motor program for walking which resulted in a more normal pattern of EMG activity during each gait cycle.

544.9 THE PHYSIOLOGY OF GAIT INITIATION. R.J. Elble, C. Moody* and C. Higgin*. Southern Illinois University School of Medicine, Springfield, IL 62794-9230. Patients with neurological disturbances of gait frequently have particular difficulty initiating a first step. We therefore undertook a study of gait initiation in eight neuromotorically normal adults, ages 18 to 87. A quick step forward was taken in response to a green light. The center of pressure of each foot was recorded with floor-mounted force plates, and surface electromyograms were recorded from tibialis anterior, peroneus longus, hamstrings, and gastrocnemius. Motion of the upper and lower extremities and torso was recorded with computerized infrared stereo photograpy. With a simple reaction time of 0.16 to 0.31 seconds, a forward step was initiated by synchronous contraction of the tibialis anterior and quadriceps, followed by a 0.06 to 0.18 seconds later, synchronous contraction occurred in the hamstrings and gastrocnemius. This stereotypic pattern of muscular activity caused the pressure of both feet to move posteriorly toward the swing foot and then ultimately toward the stance foot. These foot-floor reaction forces and muscular activity generated enough pressure from the ankles and hips that propelled the body into forward motion. (Supported by the Whitaker Foundation)

544.10 A COMPARISON IN NEUROLOGICALLY INTACT AND SPINAL CORD INJURED ANKLE JOINT COMPLIANCE AND REFLEX ACTIVITY TO ANGULAR PERTURBATIONS USING VOLITIONAL AND ELECTRICALLY STIMULATED MOVES. B.B. Flaherty1,2,3, C.J. Robinson,1,4 G.C. Aigrain5,6 and G.L. Costil1. 1Hines VA Rehabilitation R&D Center, 2Hines, IL 60431; 3Brieger & 4EEG Lab, Univ. of Illinois at Chicago; Neurology Dept, Loyola Univ., Maywood, IL; 5Physics Dept., Rush Medical College, Chicago, IL. Ankle compliance characteristics were investigated for complete (n=3) and incomplete (n=11) paraplegics and 4 intact subjects using a 10° perturbation about neutral ankle angle at various constant ankle angles. Perturbation was against a plantarflexed torque bias that was achieved via soleus stimulation. Responses were compared with those obtained via volitional plantarflexed bias in the neurologically intact. Solus and tibialis anterior surface EMG activity was recorded using an artifact-suppression simultaneous stimulation and EMG. Torque, angular displacement, and neural activity were measured and used to calculate stiffness, damping and inertial factors. During the first 100 msec of a perturbation (any speed), the compliance of the joint was the same under simulated and volitional biases, indicating that the passive properties of the joint were the predominant influence since no reflex EMG activity was seen until at least 80 msec. After 100 msec, torque became to proportional to displacement for the volitional torque bias experiments, while in the simulated case torque was not linearly related to angle. This was particularly apparent at the end of the perturbation where torque exponentially decayed to a steady state value that was significantly less than the post-perturbation torque under volitional bias. The ankle was also more with voluntary plantarflexion than with soleus stimulation, and the compliance model was more complicated with soleus stimulation. (Supported by VA Rehab. R&D Merit Review Proposal B446-R).

Dynamic transitions in stance support accompanying intentional leg flexion movements are due in part to the coordinative interaction of the ground reaction forces (GRFs) generated by the uninvolved limb. The GRFs generated by the uninvolved limb may be altered by right hemiparesis due to stroke, as was investigated in 8 adult subjects who stood on 2 separate force platforms and performed single rapid leg flexion movements with the plantar (PL) and the uninvolved (UL) limbs. The results indicated that the resultant Fy onset always preceded UL onset (+270 _ 93ms), and was responsible for the linear displacement of the body mass laterally. For UL flexion movements, however, Fy contributed a greater (p<.05) proportion (89 _ 15%) to Fy than Fxst (11 _ 15%), while PL flexion movements showed a reverse trend (p<.05) whereby Fxst and Fy became 30 _ 13% and 70 _ 12% of Fy. Strikingly, while Fxst and Fy onset times are normally coincident, 4 subjects showed a delay (140 _ 21ms) in PL Fy vs. UL Fy onset times. Moreover, in 2 cases of UL flexion movements Fxst of the PL was exerted in the opposite direction to that of Fxst of the UL so as to brake rather than assist in the weight transfer to the PL. Overall, such changes in both the timing and scaling of interlimb GRFs may contribute to lateral weight transfer regardless of the direction of the postural transition.

Supported by the Foundation For Physical Therapy.

544.13 FEATURES OF NEUROCONTROL DURING GAIT WITH REDUCED BRAIN INFLUENCE IN HUMANS WITH TRAUMATIC SPINAL CORD INJURY. J.H. Schild*, M.W. Dimitrijevic*, I. Petronic*, A.P. Sherlock. Department of Rehabilitation Neuroscience and Human Neurobiology, Baylor College of Medicine, Houston, Texas 77030.

We have studied neurocontrol in 13 ambulatory spinal cord injury subjects while they walked on a 10 meter path. We recorded bilaterally and simultaneously during gait with surface electrodes the poly- and electromyographic activity of the paraspinal, quadriceps, hamstring, tibialis anterior and triceps surae muscles. In addition, we recorded gait with force platforms and anterior, posterior, and lateral video cameras. The analysis of data during gait revealed 3 different neurocontrol features: the last enabled the neurocontrol in a subject with intact nervous system; the 2nd the patterned organized neurocontrol of flexor extensor movements; the 3rd had no organized neurocontrol.

We shall discuss how these neurocontrol patterns are generated and describe the effectiveness of different features of neurocontrol for gait.

544.15 STATIC ELBOW TORQUE-ANGLE RELATIONS IN HEMIPARETIC STROKE. J.P. Dawson, M. Monson*, T.S. Buchanan, and W.Z. Rymer, Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Illinois 60611.

In previous studies of muscle activation patterns in hemiparetic stroke, we observed limited changes in elbow muscle EMG activities during force exertions in three dimensions (flexion/extension, varus-valus, and supination/pronation). In an effort to better qualitatively characterize length-tension relations in the impaired versus the unimpaired upper extremity, we evaluated torque productions for different angles of shoulder flexion/extension abduction. Maximal forces and EMG activities were recorded at 5 angles of flexion/extension shoulder abduction ranging from -20 to 30 degrees (0 degrees = humerus aligned with the coronal plane). The angle changes only altered the length of the biceps since no other agonists cross the shoulder joint. The elbow angle was constant at 90 degrees. The vertical shoulder abduction angle was constant at 65 degrees. The forearm was maintained in the neutral supination/pronation position. These EMG values were then used to set constant submaximal activation levels by having the subject match a given fraction of the maximal EMG at each angle. Different levels of EMG activity along with the subject's RMS EMG were displayed on an oscilloscope. The subject was asked to maintain the RMS EMG at the desired level for 1.5 seconds.

On the unimpaired side, as angle changed from -20 to +30 degrees and the biceps shortened, elbow torque decreased 20 to 30%. There were no systematic differences in this present examination as a function of activation level. Similar results were observed in the impaired side. However, torque-angle relations tended to have steeper slopes in the unimpaired versus the impaired upper extremity. The degree of this difference was variable across subjects. Less steep slopes for the impaired side would be consistent with an increased in-series compliance. This work was supported by NIH grant NS-19331.

544.14 PRESENCE OF RESIDUAL MOTOR UNIT ACTIVITY UNDER VOLITIONAL CONTROL IN THE PARALYZED MUSCLES OF SPINAL CORD INJURY SUBJECTS. W.B. McKay, M.N. Dimitrijevic, M.A. Lissens*, M.A. Mici*, Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, Texas 77030.

We have carried out neurophysiological studies in 297 clinically complete spinal cord injury subjects; in 6 of them we were able to demonstrate the subclinical presence of voluntarily controlled single motor units (SMU). Three of these 6 subjects showed the activation of SMU only during particular single-joint motor tasks repetitively and consistently. Therefore, it is possible to demonstrate the preservation of a specific motor command even in subjects who have a limited number of conducting axons reaching their target.

We shall discuss how these reported results are pertinent for the understanding of neurocontrol of an isolated single-joint movement motor task as compared to the patterned, multi-joint motor task.


One of the characteristics shared by patients with depression (DEP) and closed head injury (CHI) is retardation of psychomotor performance. A central question is to what extent cognitive and motor factors contribute to the observed motor slowing.

An experimental method is presented that attempts to differentiate between cognitive and motor components underlying psychomotor retardation. Two figure-copying tasks, both differing in motor and cognitive complexity, were run. Task A consisted of tracing simple lines and task B of more complex figures. Recording of the pen movements by means of an XY-tablet (digitizer) provided objective measurements of a number of movement parameters, and made it possible to divide Movement Time (MT, time between the start and end of the drawing) in the time that the pen was on the paper (MT-down) and the time that the pen was off the paper (MT-up). MT-down was supposed to reflect motor execution and MT-up cognitive processing.

Subjects, 10 CHI patients (mean 24 yr) and 9 DEP patients (mean 50 yr), each with a matched control group, were tested on the two tasks. Both patient groups had significantly longer MT's than their controls in task B, but this was not found for the simple line drawings in task A. However, no significant difference was found between patients and controls on MT-down in task B, so the difference in MT was due to longer MT-up times for the patients. Furthermore, no interactions were found between patients and controls for motor complexity. These findings suggest that cognitive processes, rather than motor processes, are delayed. Differences in RT, errors and figure inspection are in line with these results.
545.1

FOCAL MOVEMENT VELOCITY AND PREEMPTIVE POSTURAL EFFECTS ON THE ANTERIOR POSTURAL RESPONSE. D.L. Weeks* and S.A. Wallace, Motor Behavior Lab., Ball State Univ., Muncie, IN 47306, and Dept. of Kinesiology, Univ. of Colorado, Boulder, CO 80302.

It is unclear how the motor system self-organizes anticipatory postural muscles accompanying goal-directed upper-limb movement (focusal movement) during a stratified or focal movement initiation. The present study investigated coordination of an intentional movement (from movement onset to the first measurable changes (170, 195, or 220 ms) while standing on a flat platform. Each subject adopted individual premovement postural preferences such that location of center of pressure was employed in the foot prior to movement. Each premovement center of pressure was interpreted as one component of postural muscle on set sequence as intended by the motor system to constrain the degrees of freedom present in a multi-joint task by altering postural synergies. The emergent coordination may be dependent on principles of self-organization based on the concept of pattern stability.

545.3

THE RELATIONSHIP BETWEEN AGONIST AND POSTURAL ACTIVITY IN A MICROGRAVITY ENVIRONMENT. C.S. Layne, Dept. of Kinesiology, B.S. Spooner*, Blue River Space Technologies, Kansas State University, Manhattan, Kansas 66506.

Rapid, unilateral arm raising movements are preceded by anticipatory "postural" neuromuscular activity in the back and lower limbs. Whether anticipatory postural activity is an integral component of the motor command controlling agonist activity or if agonist and anticipatory activity are independently controlled is presently not clear. A microgravity environment would presumably eliminate the functional utility of the anticipatory postural activity, thereby providing an opportunity to investigate the relationship between agonist and anticipatory activity. We used microgravity episodes during KC-135 (NASA 930) parabolic flights to collect surface EMG data from the deltoid, paraspinals and biceps femoris, during right shoulder flexions. Microgravity data was compared with baseline data collected in unit gravity. Results indicate an absence of anticipatory biceps femoris activity with anticipatory paraspinal activity remaining intact, thus suggesting biceps femoris and paraspinal activity may be components of unique postural synergies.

545.4

KINEMATIC BEHAVIOR OF HUMANS IN RESPONSE TO BASE OF SUPPORT PERTURBATIONS. D.P. Hansen and M.H. Woollacott. Motor Control Lab., Univ. of Oregon, Eugene, OR 97503.

The purpose of this study was to describe anticipatory behavior of humans to postural perturbations. To do this the kinematics of 30 subjects were examined to horizontal backward perturbations at 1.5 at 5.0/sec. Major joint movements and surface EMG from bilateral, major agonist/antagonist muscles of the legs, thigh and trunk were analyzed. Pre-perturbation subject position was controlled. Kinematic data were also obtained from a model constructed of plywood and steel proportioned to a 172 cm, 73 kg male. Ankle, hip and neck joints were modeled with hinged, and fixed spine. The model validated these findings by showing: 1. The initial ankle angle reversed occurred prior to EMG activity onset and was related to the termination of the perturbation; 2. There was a progressively increasing delay before the onset of joint movement in a caudal to rostral direction; 3. Two kinematic phases were discernable, a consistent phase (0 to 150-250 ms), and a subsequent variable phase; 4. The amount of movement decreased over successive trials. The mechanical model validated these findings by showing: 1. The joint movement reversed when the perturbation was terminated; 2. Increasing delays in joint movement onset in a caudal to rostral order; 3. The early phase's mechanical nature and the latter variable phase's CNS dependency.

545.5

CORRECTIVE REACTIONS TO NOVEL PERTURBATIONS OF THE HUMAN LOWER LIMB. W.F. McIlroy, J.D. Brooks and R.F. Collins, Neurophysiology Lab, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

It is rare to find studies which report corrective reactions to completely unanticipated perturbations. The present study uses such an approach to identify contributions, to the corrective motor responses, which are strongly determined by prior planning. Subjects (n=10) were seated on a customised, leg extension apparatus. They held control loads (200 N) with instructions to keep the slide at the starting position. Control samples, taken during each trial, acted as a precedent on which to help ensure that the first perturbation trial was completely unanticipated. A further 15 perturbation trials were later sampled at random to compare the leg extension apparatus. They held control loads (220 N) were added rapidly to cause whole limb flexion. EMG activity, in vastus medialis (VM) and tibialis anterior (TA) were recorded during the external load trials. In contrast, activity remained constant in soleus, gastrocnemius and semitendinosus. The specificity of the influence on EMG activity, seen at a frequency of 100 Hz, was observed as a significant difference in response characteristics between trials 1 and 2. Changes observed were short-term and were reflected in both tuning and triggered reactions. Systematic attenuation in TA and VM response magnitudes, from trials 1 to 16, revealed refinement of the prior plan (NSERC #A025).
545.7
THE FREQUENCY CONTENT OF HUMAN GAIT: KINESTHETIC SAGITTAL PLANE MEASUREMENTS. B. Myklebust, J. Myklebust, T. Priore, D. Kreis*, S. Saltert*. Laboratory of Sensory-Motor Performance, Zablocki VA Medical Center, Medical College of Wisconsin, and DePauw University, Marquette University, Milwaukee, WI 53295.

Sagittal plane (flexion-extension) movements of the hip, knee, and ankle of healthy adult subjects during walking have been measured using a variety of data collection methods with a range of sampling rates. Knowledge of the frequency content of movements during walking may guide future selection of equipment for data collection, appropriate choice of sampling rates, and filtering methods to smooth digital records.

We evaluated the frequency content of sagittal plane movements in level self-paced walking at 3 m/s. Data were collected simultaneously using 2 data acquisition systems: voltages from potentiometers of an electrogoniometer (Lamontsor, Orthopedic Systems, Inc.), and video data (Motion Analysis Corp.) were sampled at 60 Hz. Standard observational analysis and digitized video analysis were completed simultaneously with data acquisition from the electrogoniometer in 5 trials of right-side limb movements. Five trials of video analysis of gait without the electrogoniometer were monitored to compare the effect of the device on gait.

While joint angle profiles for electrogoniometer and digitized video analyses are similar, differences occur in regions where movements in the transverse and frontal planes cannot be corrected in the sagittal plane. Despite significant differences in sampling rates from the video analysis and the electrogoniometer, spectral analysis of both methods demonstrate that the frequency content of gait is below 5 Hz for sagittal plane movements of hip, knee, and ankle. Because sagittal plane movements in gait are represented by signals under 5 Hz, video sampling rates at 60 Hz are adequate. However, filtering methods for smoothing raw data must be used with care.


This work has been supported by research funds from VA Rehabilitation R&D.

545.9
THE EFFECTS OF DYNAMIC VISUAL ROLL STIMULATION ON SELF-MOTION PERCEPTION AND POSTURAL CONTROL. Fred H. Pravec and Thomas J. Mullen*. USAF School of Aerospace Medicine, Brooks AFB TX 78235-5301.

A widely studied visual orientation phenomenon is illusory self-motion (vection), although it may not be a good model for other visual orientation effects because it occurs at a very long latency, depends on higher-order perceptual inferences, and is cortically mediated. In this study, the latencies and magnitudes of vection and visually induced postural changes were compared using a visual roll image consisting of ~100 small white collimated squares on a wide field-of-view screen. After an initial 10-sec baseline, the image was rotated at 25 deg/sec. Subjects viewed each scene while standing on a force platform, which measured the continuous change in center-of-pressure. The latency of postural change was defined as the moment at which lateral sway first exceeded a 3-SD criterion above baseline, and was compared to vection latency. It was shown that the two measures overlapped within 2 sec, whereas vection commences on average around 7 sec. The two latency measures correlated significantly with each other, but not with the amplitude (magnitude) measures. These and previous findings imply that the experience of vection probably arises from both visual motion and a discounting of vestibular feedback over several seconds have elapsed.

(Sponsored by Air Force Office of Scientific Research).

545.11
HFCS IN BILATERAL AND IPSILATERAL SYNERGIST MUSCLE PAIRS IN HUMAN Mastication. M. Denny and A. Smith. Audiology & Speech Sciences Dept., Purdue University, West Lafayette, IN 47907.

Correlated high frequency oscillations (HFCS) observed in respiratory-related neural and EAG activity are thought to represent a widely distributed output of the respiratory CG (e.g., Bruce & Ackerson, 1986). We have proposed that bilaterally correlated HFCS observed in human mastication during mastication may represent a similar phenomenon resulting from the action of a masticatory CG (Smith & Denny, J. Neurophysiol., 1990).

If HFCS observed during chewing represent a distributed output of a CG, they should appear in ipsilateral as well as bilateral synergist muscle pairs. If correlation of HFCS increases with neural drive, interlimb correlations may be higher on the working side. Activity was recorded from jaw-closing muscles during natural chewing and chewing cycle tied for working and non-working sides. Power and coherence spectra were calculated to estimate the strength of correlation between bilateral and ipsilateral pairs. Preliminary analyses indicate that ipsilateral pairs of jaw-closing muscles show significantly correlated activity during mastication.

545.8

Phase dependent motor responses to electrocutaneous stimulation have been observed during locomotion in intact and spinal animals, and to a lesser extent in normal man (J. Yang & R. Stein, J. Neurophysiol., 45:222-3, 1981). However, human studies have not addressed the potential influence of stimulation parameters or the reaction of the contralateral limbs. This investigation was conducted to observe EMS and kinematic patterns of ipsilateral (I- and contralateral (C-0) limbs to transcutaneous posterior tibial nerve stimulation during the mid-swing and mid-stance phases of the locomotor cycle under varying stimulus conditions (force number, frequency, duration, intensity).

H-wave of the adductor hallucis muscle was monitored to judge stimulus intensity. Agonist/antagonist pairs of proximal and distal muscles were evaluated. Prominent observations were (1) enhanced I- C tibialis anterior (TA) and co-soleus (SOL), unchanged I- and TA, and increased I- swing duration when stimulation was delivered during the swing phase, and (2) unchanged TA and I- stance duration during the stance phase. Such phase dependency was most pronounced when the stimulation conditions were 3-5 pulses, 200-300 Hz, 0-5.2-Om duration, and 70% of maximum.

545.10
STABILITY OF MULTI-LOOP NEURAL CONTROL SYSTEMS. H.D. Friedman and D.B. Krasheninnikoff*. Shuttering Center Laboratory for Speech Motor Control, Department of Neurology, Baylor College of Medicine, Houston, TX 77030.

A control system can become unstable i.e. "not work" even though all its components are functioning. The instability is an emergent property of the system and occurs only when the components are connected. As Norden’s Winer observed, "The neural control systems. This means it is possible to have a neural disorder without a lesion in the classical sense. Complex nervous systems have many interactive neural loops. Following Grinn and Nashef it is useful to distinguish between anatomical, functional, and performance loops. Functional performance loops that perform functions that we propose must be accomplished to produce the observed behavior and performance are two types that are not "seen by" behavior, i.e., a muscle spindle measures the length of a muscle and feedback to the motoneuron pool of that muscle. Our model means that a system may change in a way that is not driven by a group of proposed “functional loops" that are composed of a subset of the possible anatomic loops. To consider instability we limit the system to the “functional loop that is driving a performance loop. The dynamics of the performance loop can be measured. We assume they can be approximated by a linear term and a nonlinear term and that the phase margin of the linear is skewed. Neurophysiologic tells us there will be time delays in the outer loop. These time delays effect a phase lag into the total system transfer function. This phase lag is equal to the time delay divided by the period of the sinusoidal component under consideration.

The system will become unstable whenever this phase lag equals the phase margin on the performance loop. Biological examples will be given. This work was supported by the NIH, M. Perkins, and M. Grinn.

Ariel — Benjamin — Jeremiah — Gideon — Abigail Maida Lowin Medical Research Foundations.

545.12
CONTROL OF HUMAN JAW MOVEMENT IN MASTICATION AND SPEECH. D.J. Ostry, K.G. Munhall*, J.R. Flanagan and A.G. Feldman. McGill University, Montreal, Canada and Institute for Information Transmission Problems, Moscow, USSR.

The X-ray microbeam was used to record jaw movement kinematics in the mid-sagittal plane. The jaw movements were examined in terms of the rotation of the condyle and the translation of its axis of rotation along the articular eminence. Mastication trials employed rubber tubing in which compliance and diameter was systematically varied. In speech trials, consonant-vowel combinations were produced at different rates and loudnesses. It was found that when movements of the jaw in mastication were plotted in joint coordinates, the relationship between jaw rotation and jaw translation was essentially fixed. However, when jaw movements were examined, speech relationships between rotation and translation was not constant but varied in a systematic way with the composition of the utterance. The evidence from speech suggests that the central system is capable of altering the relationship between jaw rotation and jaw translation. Microbeam recordings were also used to recover bite force during the cycle by relating structure and stimulation. The point of contact with the bolus to separately measured tension-compass functions for each of the tubes. Simulations of human jaw movement based on the equilibrium point hypothesis were found to adequately capture both free and compliant motion phases of orofacial movements. The model allows for changes in coordination between translation and rotation as well as the control of stiffness.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
545.13

This study was designed to investigate the role of motor units (MUs) in the generation of time-varying isometric muscle forces in humans. Subjects produced nearly sinusoidal contractions of the first dorsal interosseous muscle at the hand about fixed force levels (range: 15% to 55% of maximal voluntary contraction - MVC), with frequencies in the range 0.25 Hz to 5 Hz and amplitudes in the range 6% to 30% MVC. Single-MU discharges and muscle EMG were recorded from the muscle and subjected, together with the force signal, to spectral analysis. The auto-spectra of single-MU activities, as well as of multi-unit EMG, showed modulations of the discharge rates of the units, with the carrier rates (MU mean discharge rates) remaining more or less constant for a given level of contraction. This rate modulation of MU firing was at the frequency of the sinusoidal variation of the muscle force and showed a phase advance over it. The modulating component in a unit's auto-spectrum increased in size as the depth of the modulation of the force increased. Further, such modulating components were much stronger for multi-unit EMG than for single-MU discharges, which implies correlations between the modulations of different MUs. This was verified by computing covariances between the various signals, which showed large values at the common modulation frequency. Indeed, high covariances between single units, subsets of units, and the entire population signify correlations between the units.

545.15
THE FATIGABILITY OF SINGLE MOTOR UNITS IS DEPENDENT IN PART ON THE PATTERN OF ACTIVATION. Y. Laburdi, L. Bevan, J. M. Rankin and D. G. Stuart. Department of Physiology, College of Medicine, University of Arizona, Tucson, AZ 85724.

To assess motor unit (MU) fatigue, trains of constant-frequency stimuli have conventionally been used to determine the fatigue of motor neurons on their axons, either repetitively or continuously (e.g.: J. Physiol. (Lond.), 234:723, 1973; Brain Res., 327:203, 1985). However, observations of MU behavior in conscious humans suggest that their frequency of activation is not constant, even when the muscle force is constant (J. Physiol. (Lond.), 340:333, 1983; J. Exp. Biol., 115:125, 1985). Recently, we reported that MU force can be augmented during fatigue by suble changes in the activation pattern and that the efficiency of such changes increases as fatigue ensues (Neurosci. Abstr., 15:396, 1989). We now report an extension of this finding by assessing four indices of fatigue for the two activation patterns. The patterns were 300 ms in duration and delivered in random alternation at 1 Hz to the ventral root axons of 22 fast-fatigable (FF) MUs in the tibialis posterior muscle from 6 cats during a 4-min fatigue test. One pattern (regular) was comprised of a constant-frequency train (interstimulus intervals set at 1 s) at contraction time of the unit; another (irregular) pattern, the average number of stimulii, however, the initial part of this train contained a triplet (three stimuli separated by 10 ms intervals). The overall force records were decomposed to extract the force profiles attributable to each pattern (Neurosci. Abstr., 15:396, 1989). The indices of fatigue used to quantify fatigue were: 1) a progressive fatigue index; 2) a cumulative fatigue index; 3) the time to 50% of the initial force; and 4) the time to 50% of the total cumulative force-time integral. The results indicate that MUs are relatively l/s fatigued when stimulated with an optimized pattern rather than a regular pattern, irrespective of the way in which fatigue is quantified. Supported by USPHS grants NS 07309, HL 07249, NS 25077, and RR 05675.

545.16
USE OF FLUTTER FREQUENCY PERIODIC TORQUE MODULATION AS A MONITOR OF A REFLEXES DURING MOVEMENT. J.S. Thomas, Department of Physiology, Meharry Medical College, Nashville, TN 37208.

Flutter frequency (10-50 Hz) periodic torque modulation can be used as a "bounding" signal to monitor a reflex gain during ongoing movement. Averaging of sets of EMG and movement parameters, obtained with torque modulations of opposite phase relative to the TASK imperative signal (target jump and/or discrete torque change), allows unambiguous recovery of the EMG activity, and movement parameters associated with the movement task. Comparison of these "demodulated" EMG envelopes with those from the same TASK, but without periodic torque modulation, indicates that superimposed flutter frequency perturbation had NO EFFECT on TASK related performance. Subtraction of "phase180" from "phase0" data sets removes TASK specific responses and allows cycle by cycle assessment of the amplitude of EMG and movement parameter response to periodic perturbation during the TASK movement. Typically, the "vollitional" phases of TASK movement show a profound inhibition of periodic EMG modulation (assumed to reflect a reflex gain), sometimes followed by an enhancement of periodic reflex gain as the new posture is stabilized.

545.17
MOTOR UNIT ACTIVATION IS NOT TOPOGRAPHICALLY LOCALIZED IN HUMAN MUSCLE. G. Kamen, Y. Masakado*, C. J. De Luca. NeuroMuscular Research Center, Boston University, Boston, MA 01701.

In an effort to investigate the notion that muscles may be organized into functionally distinct neuromuscular compartments, the independence of motor unit action was assessed in different regions of the human tibialis anterior (TA) muscle. Motor unit recordings were obtained from the TA of five subjects using two quadrifilar needle electrodes inserted 1.5 cm apart. During isometric contractions at 30% MVC, signals obtained from each needle were decomposed to obtain individual motor unit action potential firing trains. Motor unit firing histories from within each needle site and between the two sites were studied to determine the frequency of simultaneous (synchornous) discharges, and to determine the level of common fluctuation of firing rates. These analyses indicated a high degree of synchronization at zero or near-zero latency from units recorded in both distal and proximal needle sites and a similar level of synchronous firing among units measured at both sites. Also, the fluctuation of firing rates was similar among distal, proximal and between-needle motor units, with cross-correlations of firing rate trains of about 0.6 obtained in each case. The existence of a strong central command to all motor units within a given muscle, without regard to motor unit topographical organization.

This work was sponsored by a grant from the Rehabilitation Research and Development Service of the Veterans Administration.
546.1
One consistently observed difference between the sexes concerns spatial ability. Males have generally been observed to outperform females on tasks that require them to acquire and retain spatial information. Several possible explanations for these differences have been suggested. First, males might make more efficient use of the olfactory geometric properties of the environment while females rely more heavily on information provided by single landmarks or cues. Second, males could preferentially use distal landmarks or cues when female spatial behavior is controlled primarily by proximal landmarks or cues. This notion is consistent with the ecological view that the ability of male rats to utilize distal cues more efficiently reflects the larger territory through which they roam, whereas females spend their reproductive life in a more restricted environment. The present study hypothesized that males would perform better than females on a spatial task requiring the use of distal cues whereas females would perform at least as well as males when only proximal cues were available. Forty-nine male and female Long-Evans hooded rats were tested on two versions of a water-maze task. In the proximal condition a curtain was hung just outside the perimeter of the tank. The curtain was marked with eight cues. The curtain was removed for the second condition, thus forcing subjects to rely on distal information within the larger environment. This information included cues placed around the room. There were no differences between males and females when the curtain was in place, and only proximal cues available. Males performed significantly better when the curtain was removed, and distal cues available. This advantage was not evident for the females. These results suggest that males have an advantage when the situation permits the use of distal cues, however when only proximal information is available males and females do not differ in their performance on a spatial task.

546.2
A NEURAL NETWORK APPROACH TO HIPPOCAMPAL FUNCTION IN CLASSICAL CONDITIONING. Nestor A. Schmajuk and James J. DiCarlo*. Department of Psychology, Northwestern University, Evanston, IL 60208.
We describe hippocampal participation in classical conditioning in terms of Grossberg's (1975) attentional theory. According to this theory, pairing of a conditioned stimulus (CS) with an unconditioned stimulus (US) causes both an association of the sensory representation of the CS with the US (conditioned reinforcement learning) and an association of the sensory representation of CS with the drive representation of the US (incentive motivation learning). Sensory representations compete among themselves for a limited-capacity short-term memory (STM) that is reflected in a long-term memory (LTM) storage.
We introduce the "STM regulation" hypothesis which proposes that the hippocampus controls incentive motivation, self-excitation, and competition among sensory representations thereby regulating the contents of a limited capacity STM. Under the 'STM regulation' hypothesis, we map nodes and connections in Grossberg's neural network onto a three-dimensional hippocampal circuitry. The resulting neural model provides (a) a framework for understanding the dynamics of information processing and storage in the hippocampus and cerebellum during classical conditioning of the rabbit's nictitating membrane, (b) principles for understanding the effect of different hippocampal manipulations on classical conditioning, and (c) numerous novel and testable predictions.

546.3
IMPROVED PERFORMANCE ON DRL TASKS IN RATS WITH HIPPOCAMPAL LESIONS TREATED WITH THE CALCIUM ENTRY BLOCKER, NIMODIPINE. S. Finger, L. Green, M. Moritz*, K. Mortman* and A. Anderson*. Department of Psychology, Washington University, St. Louis, MO 63130.
Rats were trained to lever press and then were given either bilateral electrolytic lesions of the hippocampus or control operations. Half of the rats in each group received oral nimodipine, while the remaining animals received a vehicle, for the 14 days following surgery. The rats were then tested on a DRL-20 sec schedule of reinforcement that required them to withhold a lever press for 20 sec in order to earn a liquid reward. Rats with lesions not given nimodipine performed very poorly while those treated with the drug performed well within the control group range. Similar trends were obtained when the rats were advanced to a DRL-40 sec schedule one month later. These findings show that nimodipine can attenuate the behavioral effects of large hippocampal lesions on even difficult memory tasks.

546.4
DIFFERENTIATION OF PASSIVE AVOIDANCE DEFICITS PRODUCED BY LESIONS OF THE CENTRAL AMYGDALOID NUCLEUS OF THE RAT. C.D. Coover and B.W. Sant*. Department of Psychology, Northern Illinois University, DeKalb, IL 60115.
Electrolytic but not ibotenate lesions placed rostrally in the central amygdaloid nucleus (rostral ACE) produce a very marked deficit in passive avoidance (PA) of drinking (Coover et al., Soc. Neurosci. Abstr., 15:1251, 1989). The present study with electrolytic lesions addresses the altered representation of the central neural contributions of the ACE nucleus, and uses an additional PA task.
Rats with rostral ACE lesions required 33.1 ± 2.9 (Mann ± SDM) footshocks of an ascending series of intensities to passively avoid drinking for 5 min. In contrast, control rats required only 18.7 ± 1.4 footshocks (p < .001). Rats with lesions aimed at the middle of the ACE nucleus (middle ACE), just 1.0 mm caudo-ventral to rostral ACE coordinates, required only 22.7 ± 1.9 footshocks, fewer than rostral ACE (p < .05) but not more than controls.
In contrast, both lesion groups exhibited deficits in a one-trial step-through PA task. Of the controls, 10 of 11 remained in the brightly lit chamber for the maximum of 10 min on the test trial one day after receiving a footshock for stepping into the dark chamber, while 0 of 8 rostral ACE and 1 of 6 middle ACE rats did so (p's < .02).
The amygdala plays some role in PA behavior, but electrolytic lesions of some portions may cause a more profound deficit for which ACE neurons aren't responsible.

546.5
SELECTIVE AND TRANSIENT BEHAVIORAL BENEFITS ARE PRODUCED BY NEURAL GRANTS THAT PROMPTLY FOLLOW RADIATION-INDUCED HYPOPLASIA OF FASCIA DENTATA GRANULE CELLS. G.A. Moklley**, J.L. Ferguson, T.J. Nemeth** and B.A. Barnett** BBS, AFPR, Bethesda, MD 20814 USA and USAFSAM, Brooks AFB TX 78259**.
X-irradiation of the neonatal rat hippocampus produces a selective hypoplasia of fascia dentata granule cells, locomotor hyperactivity, perseverative movements and deficits in passive avoidance. Transplantation of fetal hippocampal neurons into the adult (age=182±4 days) brain produced a partial behavioral recovery. (Moklley et al., Brain Res., 599:290, 1991).
Since graft/host interconnections are more prominent when transplants are conducted soon after brain damage, we transplanted hippocampal or cerebral cortex neurons when host rats were 33±5 days of age (i.e., only 16 days after radiogenic brain damage). Behavioral evaluations were conducted 80 and 182 days after grafting or surgical control procedures.
Hippocampal transplants failed to reduce deficits in passive avoidance. However, in the first test series only, selective component of locomotion (e.g., stereotypy) and perseverative turning (e.g., mean bout length and rapid turning) were improved by the grafts. These data suggest that the timing of neural graft placement may influence the pattern and duration of behavioral recovery.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990

ICl D7569 (ID) exhibited potent anticonflict activity in the rat (ED50 = 0.6 mg/kg p.o.) as well as in monkeys (ED50 = 6.25 mg/kg) procedures predictive of anxiolytic activity in man. The compound showed no effects on rotorod performance in CBA/Ca Jf mice, p.o., indicating no sedative profile. It is less potent to the sedative actions of ethanol than benzodiazepines (BDZ). In addition, the compound potent oral activity (ED50 = 1.0 mg/kg) against metrazole-induced convulsions in rats. Of particular interest, and unlike diazepam, chronic ID treatment did not cause an antagonist (80 ID15-1788) precipitated withdrawal syndrome (seizures) in mice, suggesting lack of physical dependence liability. Furthermore, ID, 10 mg/kg, p.o. antagonizes diazepam-induced sedation suggesting partial agonist activity.

Neurochemically, ID is potent (IC50 = 0.31 nM) and selective for Type 1 (cerebellar) BDZ receptor. Since, GABA increases the affinity of ID to the BDZ binding site and its potency is reduced by photoaffinity labeling, this suggests that it has agonist properties at the BDZ receptor. Thus, ID should be a potent non-sedative partial agonist anxiolytic in man with considerably reduced physical dependence liability compared to BDZ.


A variety of stressors increase extracellular dopamine (DA) and norepinephrine (NE) in the medial prefrontal cortex (mPFC) and these neurochemical responses may play a role in the etiology of clinical anxiety. We therefore assessed the effects of the anxiolytic benzodiazepine diazepam on the stress-induced release of catecholamines in mPFC. Simultaneous determination of extracellular DA and NE concentrations was performed using in vivo microdialysis in freely moving rats. Rats were given an injection of either diazepam (2.5, 12.5, or 25 mg/kg, IP) or saline and the vehicle and a later subjected to 30 min of tail- pressure stress. Diazepam alone elicited a 60-70% decrease in basal extracellular DA and NE concentrations. When this diazepam-induced shift in baseline was accounted for, tail-pressure stress elicited an 80-90% increase in DA and NE levels in both the vehicle and diazepam pre-treated rats. Thus, whereas diazepam does not eliminate the response to stress, it may exert its anxiolytic effects by decreasing the absolute magnitude of DA and/or NE release in mPFC during stress. Further experiments will examine the effects of diazepam on the stress-induced increase in NE and/or DA in the hippocampus and amygdala, sites which also have been implicated in anxiety and in the mechanism of action of anxiolytics. (Supported by an MRC of Canada Postdoctoral Fellowship to JMF, USPHS Grant MH43150 and MH43947, and a gift of diazepam from Hoffmann-La Roche.)


The primary purpose of this study was to investigate the effects of oral idazoxan, an alpha-2 antagonist agonist, on behavior and noradrenergic (NE) turnover in healthy subjects. In addition, the study sought to determine if the biochemical and behavioral effects of idazoxan are similar to those of the alpha-2 antagonist agonist yohimbine. Methods: Ten healthy male subjects received randomized, double-blind oral administration of placebo (P), 20 mg, 60 mg, 80 mg of idazoxan as well as yohimbine 20 mg, on five separate test days. Blood samples for plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) (ng/ml) and cortisol (ng/ml) and behavioral ratings were obtained at baseline and at intervals for up to 4 hrs following the oral study dose. Results: The 20 mg (1.3±0.8, p<0.01), 40 mg (1.2±0.95, p<0.003), and 80 mg (2.0±1.34, p<0.001) dose of idazoxan resulted in consistent, significant increases in plasma free MHPG. The 20 mg dose of yohimbine also resulted in a significant increase in plasma free MHPG (1.7±0.12, p<0.002). Plasma cortisol levels were significantly increased following the 80 mg dose of idazoxan (3.5±2.7, p<0.05) and following the 20 mg dose of yohimbine (4.6±1.5, p<0.05). Systolic and diastolic blood pressure (sitting and standing) increased significantly following all three idazoxan doses and following yohimbine. Conclusion: The robust increase in plasma MHPG following all three doses of oral idazoxan demonstrates that idazoxan increases NE turnover in human subjects. Although idazoxan and yohimbine may differ in their selectivity for alpha-2 receptor subtypes, this study suggests that neuroendocrine and behavioral responses to these drugs are similar in human subjects. The measurement of the effect of idazoxan on NE turnover and behavior may provide a means of assessing alpha-2 antagonist agonist function in humans.

AUTORADIOGRAPHIC DISTRIBUTION OF 5-HT1A RECEPTOR BINDING SITES FOLLOWING SUBCHRONIC TREATMENT WITH IPSAPRINE. R. McMonagle-Strucko, B.R. Feinelli, Institute for Preclinical Pharmacology, Miles Inc., West Haven, CT 06516.

Ipsapirine has been shown to have potent anxiolytic and antidepressant properties in a variety of animal models. Ipsapirine, a high affinity ligand for the 5-HT1A receptor subtype, is a full agonist at presynaptic afferent and efferent sites and a partial agonist at postsynaptic sites. Experimental data are now available indicating whether treatment with ipsapirine would differentially affect binding to 5-HT1A receptors at these different sites and whether the effects are dose-related. In mice, rats, and hamsters treated twice daily with ipsapirine (10 mg/kg ip) for 14 days. Quantitative analyses were done of autoradiograms of in vitro brain sections (CNS, with or without 10 µM 5-HT, as opposed to [3H] serotonin-sensitive film for 5 weeks) to selected brain regions. Binding in untreated rats was highest in the hippocampus, septum, entorhinal cortex and raphé nuclei. Subchronic ipsapirine treatment resulted in a large decline (50%) in binding in raphé nuclei and to a lesser extent in the entorhinal cortex, without altering other regions analyzed. These data support the hypothesis that region specific effects of ipsapirine contribute to its behavioral profile.
547.7
THE 5-HT1A AGONIST, 8-OH-DPAT, STIMULATES FOOD INTAKE OF RHEUS MONKEYS. S.M. Pomponey, Dept. of Physiology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

There is a growing interest in evaluating pharmacological agents which may be clinically applied in the treatment of eating disorders. In the present study, we developed a simple feeding paradigm to be used in primate species to examine the effects of pharmacological manipulation on food intake. Adult male rhesus monkeys were placed on a feeding schedule in which a main meal of Purina monkey chow was provided at 1000 hr and a supplemental meal of monkey chow was provided another time during the day. Monkeys generally ate most of their food during the first two hours following their main meal, but also "snacked" following supplemental food presentation. This supplemental feeding period was used to evaluate the effects of the 5-HT1a agonist, 8-OH-DPAT (DPAT).

DPAT (10 µg/kg) increased food intake when administered at either 2 hr or 6 hr after the main meal, but did not when administered immediately prior to the main meal. Facilitation of supplemental food intake by DPAT was most prominent during the first hour after its administration, but by 4 hours after its administration, food intake returned to control levels. Dose-response analysis revealed that DPAT exhibited a potent biphasic effect during the first hour after its administration (m=7), with low doses (5, 10, and 25 µg/kg) reliably stimulating food intake 166, 208, and 212%, respectively over control vehicle levels, and a high dose of DPAT (200 µg/kg) inhibiting food intake to 51% of control levels. These results indicate that pharmacological agents that interact with 5-HT1a receptors influence food intake in a primate species and that such agents may be clinically efficacious in humans in controlling food intake.

547.8

Acute administration of gepirone, a 5-HT1a agonist, produces anxiolytic-like effects in mice and rats. Gepirone also produces a treatment duration-dependent anxiolytic-like effect that persists for up to four days following its elimination. Our study was designed to characterize the alterations in serotonergic receptors which are produced by sustained treatment with gepirone, and which are related to the onset and continuation of the anxiolytic-like effect. Mice were injected chronically with gepirone, 15 mg/kg, bid, and after discontinuation of the treatment, performance on behavioral tests, and in situ receptor binding were assessed. In the gepirone-treated mice, a significant anxiolytic-like effect as revealed by specific performance in the elevated plus-maze was observed at 24-96 hours following 7, 14 or 21 d of treatment. There was a significant decrease (up to 50%) in the density of cortical 5-HT1a receptors. No change was detected in 5-HT1b receptors or in amine release sites; 5-HT1a binding increased, but this effect was preceded by the behavioral changes. Head-shakes induced in mice by 1-25 dimethyl-4-iodophenyl-3-aminopropane (DOI; 1mg/kg), thought to reflect 5-HT1a receptor function, were virtually abolished following 7, 14 and 21 d of gepirone treatment, but not after 1 d of treatment. 5-HT1a receptor down regulation and decrements in the head-shake response to DOI were correlated with the anxiolytic-like effect, thus suggesting that decrements in the density and function of 5-HT1a receptors underlie the anxiolytic-like effects of gepirone. Supported partially by NIAAA grant ROI 3521.

547.9
INTERACTION OF ADRENORECEPTOR ANTAGONISTS WITH 5-HT1A AGONISTS IN DRUG DISCRIMINATION. L. Zhang and J. B. Barrett, Dept. of Psychiatry, Uniformed Services Univ. of the Health Science, Bethesda, Maryland 20814.

In order to investigate the mechanism of action of novel anxiolytic drugs such as buspirone, the beta- and alpha-adrenoceptor blocking drugs, pindolol and prazosin were studied in a drug discrimination procedure. Pigeons were trained to discriminate 8-OH-DPAT (0.3 mg/kg) from saline. Dose-response curves were tested with the drugs before and after chronic administration of 8-OH-DPAT (3.0 mg/kg/day for 4 to 6 weeks). Dose-response curves for 8-OH-DPAT and buspirone were shifted to the right after chronic administration. Prazosin did not generalize before or after chronic injection. Pindolol did not produce drug key response change before but it did so after chronic administration of 8-OH-DPAT. Although some report that pindolol has 5-HT receptor antagonist effects, it may have partial 5-HT1a receptor agonist-like properties. After 8-OH-DPAT chronic administration, these receptors may be more sensitive to pindolol. The results indicate that alpha-1 antagonist properties are not directly involved in mediating discrimination stimulus effects of 8-OH-DPAT. 8-OH-DPAT and the beta-adrenoceptor blocking agent pindolol can share discriminative properties under certain conditions. Our approach may provide a useful behavioral model to investigate the mechanism of anxiolytic effects of 5-HT1a agonist. (Supported by DA-02873).

547.10
REGION-SPECIFIC TOLERANCE TO THE 5-HT1A AGONIST IPSAPRINE. M.F. Piercey, Y. Tian*, J.T. Lumin, W.E. Hoffmann, R.J. Collins*, M.M. Cooper*, K. Lookingland, and K.E. Moore. The Upjohn Company, Kalamazoo, MI USA and Michigan State University, E. Lansing, MI USA.

In order to better understand delayed therapeutic onsets of 5-HT1a anxiolytics, we evaluated acute ipsapirine (IPS) in animals treated with chronic IPS (15 mg/kg/day iv, infusion, 7 days). In chronic saline-animals, IPS depressed 5-HT neuron firing (dorsal raphe, EDa = 12 µg/kg i.v.), 5-HIAA/5-HT ratios, body temperature and regional brain energy metabolism (2-DG autoradiography), especially in hippocampus, cortex, basal forebrain and raphe areas. In chronic IPS-pretreated animals, the chronic drug was less potent in depressing 5-HT neuron firing (EDa = 53 µg/kg) and slightly less effective in producing hypothermia. Tolerance was observed to IPS's depression of 5-HIAA/5-HT ratios in the hippocampus, but results were inconclusive elsewhere. In 2-DG autoradiography, acute effects were depressed by chronic treatment in some regions (e.g. prefrontal cortex, m. hypothalamus, basal forebrain, etc.) without being significantly altered. In studying the hippocampus, it is concluded that tolerance differentially develops among brain regions and this could be relevant to the delays observed in anxiolytic activities of the drug.

547.11
EFFECTS OF &bgr;-ADRENORECEPTOR ANTAGONISTS ON SEROTONIN METABOLISM AND AGGRESSION IN ISOLATED MICE. J.K. Chamberlain and J.P. Davanzo. Department of Pharmacology School of Medicine, East Carolina University, Greenville, NC 27838.

&bgr;-adrenoceptor antagonists are known to inhibit isolation-induced fighting behavior in mice. Receptor binding and pharmacologic effects in vivo with affinity for serotonin (5-HT)&micro; receptors and not &bgr;-receptors. Activation of 5-HT1A receptors is known to decrease serotonin impulse flow and the &bgr;-adrenoceptor antagonist prazolam is known to antagonize this effect. The effect of acute and chronic &bgr;-antagonist administration on 5-HT metabolism was examined isolated feeding mice, behaviorally, 8,1-biphenyl, significantly increased 5-HT levels in olfactory bulbs and hippocampus. Administration for ten days had no effect on meal counts, but the diametrical acid (5-HIAA) was increased in septum and hippocampus of mice treated with 8,1-biphenyl. Prazolam and prazolam, and 8,1-biphenyl at behaviorally effective doses had no effect on 5-HT or 5-HIAA levels in any group tested. Since decreased serotonin activity is associated with decreased aggression, increased serotonin metabolism as indicated by increased 5-HIAA levels is probably not the mechanism by which &bgr;-antagonists inhibit fighting behavior. (Supported by Hoechst-Roussel Pharmaceuticals Inc.)

547.12
SIGNIFICANT SEDATIVE EFFECTS REPORTED WITH TWO PUTATIVE NONSEATIVE ANXIOLYTIC DRUGS USING THE ACCELERATING ROTOROD. R. C. Meyer, C. E. Lints and W. J. Krasnow. Department of Psychology, Northern Illinois University, belaeb, IL 60115.

Previous research with the drugs buspirone and premaze-pan has demonstrated a lack of sedative properties with these agents (as measured by inclined screen test, wire-grasping test, and constant rotation rotorod), and hence they have been referred to as putative nonselective anxiolytics (PNA). This is in contrast to benzodiazepines such as diazepam which, although anxiolytic, possesses sedative side effects.

The present study investigated the sedative properties of buspirone, premaze-pan, and diazepam compared to vehicle controls in female Swiss-Tibino mice (P<10/drug group) using the accelerating rotorod. Mice injected with a drug were placed on the rotorod, which slowly accelerated from 4-rpm to 40-rpm over a 5-min session. The latency (sec) to fall off the rotord was recorded. Buspirone (1.25, 2.5 and 5 µg/kg), premaze-pan (10 and 20 mg/kg), and diazepam (1.25, 2.5 and 5 mg/kg) all produced significantly shorter fall-off latencies than controls in a dose-dependent manner. The results suggest that the accelerating rotorod may provide a more sensitive test for measuring the sedative properties of drugs than most experimental techniques currently in use, and also question the validity of classifying buspirone and premaze-pan as PNAS.

Contributions of the $\alpha_2$ adrenergic and DA systems to certain pharmacological effects of B-HT 920 were investigated. Male C57 Bl/6 mice (Simonsen) were used and all injections were made i.p. The profound hypothermic response to B-HT 920 (0.1-0.5 mg/kg) was reversed by haloperidol (0.5 mg/kg), but not by idazoxan (1.0-3.0 mg/kg). B-HT 920 (0.1-1.0 mg/kg) prolonged the duration of ethanol (3.0 g/kg) induced loss of the righting reflex in mice. Pretreatment with idazoxan (1.0-3.0 mg/kg) fully prevented this effect of B-HT 920. B-HT 920 (0.3-1.0 mg/kg) reduced the locomotor activity and righting reflex in mice. Even though idazoxan (1.0-3.0 mg/kg) itself reduced the d-amphetamine-induced hyperactivity, pretreatment with this $\alpha_2$ antagonist prevented the B-HT 920-induced blockade of the amphetamine effect. It was concluded that while the hypothermic effect of B-HT 920 resulted from post synaptic DA receptor stimulation, the other two pharmacological effects are related to its $\alpha_2$-adrenergic agonistic effects (Supported by the U.S. Veterans Administration).

547.15 EXOGENOUS TYROSINE POTENTIATES THE METHYLPHENIDATE-INDUCED RELEASE OF Dopamine in the NUCLEUS ACCUMBENS as MEASURED by IN VIVO Microdialysis. S.K. Woods and J.S. Mayer, Dept. of Psychology, University of Maine, Orono, ME 04469.

The synthesis and release of dopamine (DA) may, under certain conditions, be altered by increased availability of its amino acid precursor, tyrosine (TYR). To examine whether exogenously supplied TYR could potentiate the release of DA induced by methylphenidate (MPD), 7 Sprague-Dawley rats 4 months of age were implanted with microdialysis probes in the n. accumbens. Samples were collected from awake, freely moving animals beginning 24-24 h after surgery. Twenty-microliter 3-MP (0.4 mg/kg) was administered i.p. twice at 6 h intervals for 4 days, once a day for 3 consecutive days in a repeated measures design. On a given day, the animal was infused with 30 microliters of MPD or 30 microliters of saline (controls). No apparent effects of TYR were observed on the tissue levels of DA or on the release of DA in the artificial CSF. Periods of infusion with the active compound(s) were preceded and followed by baseline conditions and treatments were counterbalanced to control for possible order effects. MPD plus TYR significantly increased extracellular levels of DA compared to drug alone. This effect was long-lasting, persisting into the post-treatment period and peaking 40 min after the peak induced by MPD alone. TYR alone induced a small but steady rise in extracellular DA that did not reach significance until the time of the first post-treatment sample. These results have implications for the use of TYR along with MPD in the treatment of attention deficit disorder.


The non-opioid antitussive dextromethorphan (DM) and PCB/sigma ligands such as (+)-SKF 10047 (SKF10047) and (+)-3-PPP appear to share a common binding site but display different pharmacological profiles in vivo. We suggested that DM may be a functional antagonist at these sites. Using an animal model of EEG and behavioral rat model, we previously demonstrated that pretreatment with DM antagonized the dual peak EEG spectral profile of (+)-3-PPP (Tortella and Robles, 89). In the present study, we assessed the ability of DM to alter the anticonvulsant effect of the PCB/sigma ligand (+)-SKF10047, and the proconvulsant effect of the selective sigma ligand (+)-3-PPP, in the rat flurorothyl test. In this seizure threshold (ST) test, doses of DM alone up to 25 mg/kg (s.c.) were inactive. However, pretreatment with 25 mg/kg DM significantly attenuated the (+)-SKF10047-induced increase in ST and the (+)-3-PPP-induced lowering in ST. The maximal anticonvulsant and proconvulsant effects of (+)-3-PPP, in the flurorothyl test, were antagonized by 38% and 79%, respectively. Importantly, increasing the pretreatment dose of DM from 25 to 50 mg/kg (s.c.), was sufficient to overcome the "antagonist" effects of DM, suggesting that the interaction between these ligands is competitive and reversible.


This study assessed acoustic startle reflex (ASR) as a screening test for centrally-acting muscle relaxants (CMRs). ED50s were calculated for inhibition of ASR (25-200 Hz) by morphine (MPD) and d-amphetamine (MPD). ASR was calculated as the percentage of the mean absolute ASR (30 mg/kg/morphine salinate SG) in male, Wistar rats. ED50s for ASR were 6.25 mg/kg, 180 mg/kg, and 30 mg/kg for MPD, (+)-SKF10047, and (+)-3-PPP, respectively. ED50s for ASR were calculated for 8 CMRs. CinDexide, chlorpromazine, methadone, and cyclobenzaprine antagonized rigidity and inhibited ASR with roughly equivalent ED50s (ratios = 0.9 – 1.1). Diazepam and carisoprodol antagonized rigidity more potently than they inhibited ASR; ratios were 1.7 and 1.9, respectively. The CMRs with ratios of 0.9 or higher are effective for the treatment of muscle spasm associated with exertion or strain. Baclofen and tizanidine (ratios 0.6 and 0.2 respectively) inhibited ASR more potently than they did antispasticity. These two drugs are used to treat spasticity associated with spinal cord lesion or cerebral palsy. The differences in potency of the drug tested in determining the clinical indication for which potential CMRs are best suited.

Pulse-type electric fish are able to localize the source of an electric field, such as that produced by a conspecific, by aligning their body long axis parallel to the local electric field and following the current lines until they reach the source. We are interested in the sensory cues that the fish use to determine this alignment error. We recorded from >150 afferents from tuberous electroreceptors in the gantry of the anterior lateral line nerve in fish suspended at the center of a large circular tank. Stimuli mimicking the electric organ discharge (EOD) were presented through pairs of electrodes spaced at 15° intervals around the tank perimeter. We precisely located each receptor on the body surface using a monoprobe.

Variations in stimulus angle cause systematic variations in the responses of both burst duration coder and pulse marker afferents. Polar plots of spike number, latency, and threshold all have two elliptical lobes oriented 45° apart. The lobes are often markedly asymmetrical with the major lobe pointed into the fish. The "best" angle varies systematically over the body surface with pulse markers and burst duration coders in the same area showing similar preferences. Units on the snout, tail, and dorsal midline prefer fields parallel to the body axis, while units located on the flank, especially over the thickest part of the body, respond best to transverse fields. The greatest overall sensitivity to any stimulus angle is found on the head; the lowest is found on the tail.

Our data establishes that information is available to the CNS to permit left versus right and head versus tail comparisons as a possible basis for correcting orientation errors. Supported by NIMH grant # ROI MH 37972 and NSF grant # BNS 8810809.

548.2 DIRECTIONAL CHARACTERISTICS OF TUBEROUS ELECTRORECEPTORS IN ELECTRIC FISH, HYPOTHALAMUS SP. D.D. Yager and C.D. Hopkins. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Pulse-type electric fish are able to localize the source of an electric field, such as that produced by a conspecific, by aligning their body long axis parallel to the local electric field and following the current lines until they reach the source. We are interested in the sensory cues that the fish use to determine this alignment error. We recorded from >150 afferents from tuberous electroreceptors in the gantry of the anterior lateral line nerve in fish suspended at the center of a large circular tank. Stimuli mimicking the electric organ discharge (EOD) were presented through pairs of electrodes spaced at 15° intervals around the tank perimeter. We precisely located each receptor on the body surface using a monoprobe.

Variations in stimulus angle cause systematic variations in the responses of both burst duration coder and pulse marker afferents. Polar plots of spike number, latency, and threshold all have two elliptical lobes oriented 45° apart. The lobes are often markedly asymmetrical with the major lobe pointed into the fish. The "best" angle varies systematically over the body surface with pulse markers and burst duration coders in the same area showing similar preferences. Units on the snout, tail, and dorsal midline prefer fields parallel to the body axis, while units located on the flank, especially over the thickest part of the body, respond best to transverse fields. The greatest overall sensitivity to any stimulus angle is found on the head; the lowest is found on the tail.

Our data establishes that information is available to the CNS to permit left versus right and head versus tail comparisons as a possible basis for correcting orientation errors. Supported by NIMH grant # ROI MH 37972 and NSF grant # BNS 8810809.

Mormyromast electric receptors are the most numerous type of electric organ in mormyrid fish and are responsible for active electrolocation. Little is known about the relative contributions of information from these electric receptors. Single cells were therefore recorded in the mormyromast region of the electroreceptive lobe, the first central relay of the system. The effects of local electrostimulation to the skin and of the electric organ discharge (EOD) motor command were examined with extracellular recording.

Three major categories of cells were found: 1) cells excited by the EOD command and inhibited by electrostimulation; 2) cells in which the EOD command had only moderate effects in isolation but strongly facilitated an excitatory response to a stimulus; 3) cells inhibited by the EOD command and excited by electrostimulation. Distinct subtypes were present within the first two categories.

The effects of the EOD motor command were modifiable and depended on previous pairing of the command with a sensory stimulus. The direction of modification was to oppose the effect of the paired stimulus. Two types of modification in command effect were evident; a large modification occurring over several minutes, and a small modification occurring over a few seconds.

The slow modification is similar to that described previously in the ampullary region of the electroreceptive lobe and could serve in adjusting the system to slow environmental changes. The fast modification was not observed previously and could serve in the active electrolocation of rapidly changing events.

548.6 ELECTROSENSORY MODULATION OF ESCAPE. J.G. Canfield and J.G. Rose, Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

Using a sudden-onset pressure stimulus, we show that the weakly electric fish Eigenmannia maintains a low threshold to startle, due to a delay of effects a discharge, and easily avoids obstacles regardless of ambient light levels. These fish also suggest that some maneuvers through the electric field mimicking the presence of a conspecific.

In contrast, the highly visual goldfish Cistoneus, easily avoid obstacles and have a diverse range of maneuvers only when pressure discharge is present, and usually when the animal is actively swimming through the tank. Escape rarely occurs when goldfish is in a "freezing" position.

In dim light, dis. But response is easily "avoided" though the magnitude and trajectory of the responses are highly restricted. (Canfield and Metzner, Brain, Behav. Evol., 1990) "Freezing" behavior is an ethologically important strategy for maintaining concealment and optimal detection of predators.

Physiological evidence obtained from Eigenmannia suggests that the above Behavioral data. While the Mauthner nerve cell does not appear to receive electroreceptor inputs directly, such inputs have been recorded in other reticular formation cells. The spike activity of these cells is influenced by a mimic of the fish's own electric organ discharge. These puffin "nudibranch" escape-modulating brainstem neurons (Eaton et al., Brain Behav. Evol., 1989) may be specifically linked to the electric fish. The ability of Eigenmannia to maintain robust escape responses and avoid obstacles in a three-dimensional environment appears to be related to its electroreceptor abilities. Supported by NSF and the Sloan Foundation.

548.7 DISCRIMINATION OF THE SIGN OF FREQUENCY DIFFERENCES BY THE WEAKLY ELECTRIC FISH, STERNOPYGUS. G.J. Rose and J.G. Canfield, Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

In its "Jamming Avoidance Response", the gymnotiform electric fish, Eigenmannia, shifts the frequency of its electric organ discharges (EOD) away from that of a "jamming" signal. The fish is able to correctly determine the sign of the frequency difference (DF) between its EOD and the jamming signal. Behavioral and electrophysiological studies indicate that Eigenmannia determines the sign of DF by a complex analysis of the temporal relationship between modulations of the amplitude and phase of the signals received by different areas of the body surface.

To gain insight into the evolution of this behavior, studies were conducted on fish of the related genus, Sternopygus. An associative conditioning paradigm was developed to determine if Sternopygus, despite lacking a JAR, is able to discriminate the sign of DF. Movement of a "nuisance" object direction was increased by presentation of a signal slightly higher in frequency than that of the fish's EOD, while a signal slightly lower in frequency was paired with the opposite direction of movement. Both species of Sternopygus were able to learn this discrimination within 1000 trials. Evidence will be presented supporting the notion that these fish achieve this discrimination by the same mechanism as does Eigenmannia and that they possess modulation filters. Supported by grants from NSF and the Sloan Foundation.

548.8 NEURAL CODING AND PROCESSING OF COMMUTATORY SIGNALS IN THE ELECTRIC FISH, EIGENMANNIA. U. Metzner * and N. Heiligenberg, SIO, UCSD, La Jolla, CA 92039.

Eigenmannia produces continual, nearly sinusoidal electric organ discharges (EODs) and has two classes of electro receptors, ampullary units which code low-frequency signals (0 - 40Hz), and tuberous units which are tuned to the fish's fundamental EOD frequency (200 - 500Hz). Separate structures process the inputs from these two receptor classes in the electroreceptor and neurophysiological studies of Eigenmannia indicate that frequency of spikes is used for this frequency discrimination. The same sensory information is used in the detection of objects and in the avoidance of objects. The frequency of spikes is used to detect objects and in the avoidance of objects.

548.9 DIFFERENT CLASSES OF GLUTAMATE RECEPTORS MEDIATE DISTINCT MODULATIONS OF THE MEDULLARY PACEMAKER OF A GYMNOTIFORM ELECTRIC FISH. Masashi Kawasaki AND Walter Heiligenberg. UCSD, La Jolla, CA 92039.

Gymnotiform electric fish generate distinct communicatory signals by modulating the rate of their electric organ discharges (EODs). Each EOD is triggered by a command pulse from the medullary pacemaker nucleus which contains pacemaker cells and relay cells. A sudden rise in the rate of this nucleus is modulated by inputs from the diencephalic prepacemaker nucleus. The pharmacological separation of behaviors previously found in E. Eigenmannia has been made in the "pulse"genic Hypopomus. The NMDA receptor blocker APV and the kainate/glutamate receptor blocker CNQX, administered to the prepacemaker nucleus, abolished the frequency of "beats", "chirps", respectively, indicating that different classes of glutamate receptors mediate the generation of different frequency signals. APV, in a form of sustained modulations, the "sudden interruption", appears to be mediated by NMDA receptors as well. Despite the rather different frequencies of electric organ discharges observed in these genera, it appears to be a common feature that NMDA receptors are used for sustained modulations, whereas kainate/glutamate receptors mediate rapid modulations.

548.10 CONDUCTANCES CONTRIBUTING TO THE ACTION POTENTIAL WAVEFORM OF ELECTROCYTES IN STERNOPYGUS. M.B. Ferrari and H.H. Zelen, Dept. of Zoology, Univ. of Texas, Austin, Texas, 78712.

Electrocyte spike duration (ESD) in this species varies with the electric organ discharge frequency. The ESD is longer in males than in females and is modulated by somatic hormones. A current clamp study was used to investigate membrane properties using blockers and tetanic substitutions to determine the conductances (g's) which contribute to the shaping of the spike waveform (SPWF). The average resting potential was -449 mV, with a range of -111 mV (n=62). The average overshoot amp. was 18.75 +/-.16.59mV (n=41). A complete but reversible block of the spike occurred in 1.25mM TTX. In addition, A5, which blocks sodium inactivation, resulted in a dramatic increase in ESD (amp. 5.05 to 14.35mV and +17mV amp in one case). Cao g's, however, do not appear to contribute significantly to the ESD and may be more related to the generation of the wavefront than to the generation of the "beats". The TEA effects were also reversible. The application of 1-4M-AP, however, caused a dramatic increase in spike amp. and in an even faster rise time than that observed with TEA. The effect of 4- AP on the phase angle also appeared qualitatively different than that of TEA. These results suggest the presence of at least two separate K+ g's. An increase in g during hyperpolarization, was also blocked and was prevented by both 0.1mM CoCl2 and 4-AP. A pre-pulse results in a spike onset delay during the depop, pulse, implicating an A-type cond. and/or an inward rectifier in shaping the rising phase of the spike. The results to date are consistent with a +4 spike "beats", a K+ g's an inward rectifier, an A-type g, and a delayed rectifier. Supported by NHI.
548.11
BROMODEOXYURIDINE LABELLING OF REGENERATING ELECTRIC ORGAN REVEALS A CLASS OF SATELITE- LIKE CELLS, J. M. Patterson, and H. H. Zelen, Dep. of Zoology, University of Texas at Austin, Austin, TX, 78712. 

Weakly electric fish of South America, the gymnotiforms, are a highly regenerative vertebrate species. Many species regenerate their posterior body parts, including muscle, connective tissue (CT), and electroreceptor complexes (ECs) of the electric organ discharge (EOD). In Stenopterygii, the EOD is made up of long (1000 μm), cylindrical cells which have been suggested to be of myogenetic origin. EMD micrographs of EOD reveal small satellite-like cells around the perimeter of the EC embedded in the extracellular matrix. We have studied these cells using the bromodeoxyuridine (BrdU) labelling technique and found that they divide and proliferate after EOD amputation. 

New Stenopterygii marcus was a 10-20 mm segment of the tail posterior to the anal fin repaired and reconnected with either the Blot Roof or saline after 2, 4, and 6 days. After a 3 hr, labelling period, the proximal stump was fixed, frozen sections cut, incubated with anti-BrdU, Ab, and visualized with fluorescent or HRP-conjugated mouse secondary. 

Two days post-amputation (n=3), satellite-like cells close to the cut were labelled, as well as epidermal cells. At day 6 (n=3), there is extensive labelling of satellite-like cells near the wound margin, and at sites several hundred microns proximal to the cut. We observe an aggregation of rapidly dividing cells near the wound margin, and a large cluster containing many labelled cells is present. No labelling was seen in any area of the lateral line. These cells may be a previously unreported pool of satellite cells within the electric organ, and that division of these cells may contribute to the formation of the regeneration blastema. Supported by NIH.

548.13

A. leptohynchus is a weakly electric gymniform fish with a high frequency (600 to 1000 Hz) wave-type electric organ discharge (EOD). Previous measurements of the EOD in the midplane distant from the fish have shown an approximate dipole field with poles centered in the trunk and tail. In the current experiments, we have mapped the electric potential and electric field components of the EOD adjacent to the skin at numerous horizontal and longitudinal planes on the side of the fish. Recordings from different positions were synchronized by an EOD phase reference measured at a stationary electrode, which allowed the precise temporal structure of the EOD to be determined. Electric field components to the fish body were calculated. Results reveal a more complex EOD portrait than that seen with far field measurements, especially in the tail region where complex phase relationships were found. 

These provide important constraints for our attempts to model both the generation of the EOD and the central analysis of electrosensory data. With respect to the electric organ, our data suggests the electrosensors do not fire synchronously. But the stability of the EOD waveform over multiple periods suggests that the relative phase of firing of these electrosensors is tightly controlled. With respect to the processing of electrosensory information, our results indicate that the tubers electrosensors found in the tail region experience a considerably different electrical environment than those in the trunk and head. In addition to complex phase relationships, field amplitudes near the tail are an order of magnitude larger than over the trunk. Since the sensory field is highly sensitive to tail position, the fish must either control this parameter precisely, and/or compensate for its effects. These results suggest that information from the caudal electrosensors may be used differently in central processing. Supported by an NIH NIDR grant (RR07003), and NIH and ONR postdoctoral training grants.

548.15
AUDITORY NEUROPHYSIOLOGY OF THE MESENCEPHALON IN THE HUMPBACK WHALE, J.D. Crawford, R. E. Fay, * Department of Hearing Institute, Loyola U., 6525 N. Sheridan Rd, Chicago, IL 60666.

We are interested in temporal processing and the neural representation of complex sounds. We are studying sounds because these are temporarily-patterned sounds in communication (Crawford et al., 1986). They have peripheral gas bellows for sound pressure transduction at the suckle, and their auditory pathways are known anatomically. Here we summarize responses of single neurons, in nucleus Mediales Dorsalis (nMD), stimulated with clicks presented under water.

We encountered neurons that gave either phasic responses or tonic responses to tones. Most had loud spontaneous activity (c. 1.0 spikes/s) and responded with short latencies (5 ms). Thresholds were ~30 to ~13 dB r.e. 1.0 dyne/cm² (communication signals are ~30 dB at 10 cm). Characteristic frequency bands (CFs) of typical CFs were ~235 Hz and this is very close to the 220 Hz fundamental of a nearly tonal signal produced by the whale. Some of these tonic neurons exhibited sharp frequency selectivity (Q 2.5). The CFs of phasic neurons have also been primarily in the 235 Hz range but some had higher CFs between 400 & 500 Hz and all were briefly tuned (Q 2.5). 

All phasic neurons showed tight synchronization to clicks during trains with intervals of 10 to 60 ms, a range that included intervals characteristic of two pulsatile sounds also used in communication. Many nMD neurons had non-monotonic rate-intensity functions with a peak at about +5 dB. We have also observed phasic responses to some nononoic responses and responses 40 ms after tone bursts, but only at relatively high levels (e.g. 20 dB). We are currently investigating the possible role of inhibition in blunting these ERPs.

These response properties are consistent with the idea that nMD functions in processing of complex communication (NIH NRSA DC00205-02 & CDR P50 DC00293-06).

548.16
LHRH-POSITIVE PROCEPTIC CELLS IN A SEX REVERSING FISH: SEXUAL MORPHOPHYSIOLOGY AND MODULATION OF GONADAL STEROID HORMONES. M. S. Grober and A. Bax, * Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

The sex-reversing fish, Thalassoma bifasciatum, exhibits two color morphs (primary phase, PB, and terminal phase, TP). PB fish are born either male or female, while TP males result from the transformation of PP males or females. Previously, we identified LHRH-like immunoreactive cells in the preoptic area (POA) of this species. This analysis provides a quantitative comparison of LHRH POA cells amongst the different sexual phases and addresses the role of gonadal steroid hormones in regulating cell number. 

Total LHRH POA cell number was 2-3 times higher in TP males versus PP males or females, which were similar to each other. There was no significant difference in the size of LHRH POA cells between either sex of TP males and females. The total number of LHRH POA cells was greater in both PP males and females, intrapituitary implants of 11-Ketotestosterone, a naturally occurring gonadal androgen, induced significant increases in the number of LHRH POA cells to levels observed in females and males. There was, however, no significant effect of androgen implants on either cell number in TP males, or cell size in any of the sexual phases. Finally, all satel treated PP fish assumed TP coloration. These results demonstrate a naturally occurring sexual polymorphism in LHRH POA cell number whose expression is influenced by gonadal steroid hormones. Given the known role of the POA in the control of reproductive function, LHRH POA cell number may reflect a sexual or reproductive behavior that is characteristic of the different sexual phases in sex reversing fishes. Supported by a NIH Postdoctoral Training Grant (MSG) and NIH/NSF Grants (AB).
548.17


Male and female toadfish (Opomus taui) produce an agonistic grunt call by contracting a pair of sonic muscles intrinsic to the swim bladder. The swim bladder and sonic muscles increase in size for life and are both larger in males than in females. In this study we investigated the effect of fish size and sex on spontaneous grunts recorded in air. Fundamental frequency ranged between 120 and 160 Hz and did not vary with fish size or sex, confirming that the frequency spectrum is determined by muscle contraction rate and not swim bladder size. Individual grunt trains varied from 3 to more than 30 grunts, and grunt durations varied from 13 to over 200 ms. In a long series, durations tended to decrease and intergrunt intervals to increase with time. Although all fish were recorded under identical conditions, SPLs within a train ranged between 3-13 dB and were similar in males and females. Although the toadfish sonic system is thought of as all-or-none, this variation suggests that the fish can recruit different numbers of motor units. Maximum SPLs were approximately 70 dB re. 20Pa at 20 cm in males and females and tended to increase with fish size.

548.18


We are using simultaneous electromyographic (EMG) recording and high speed image acquisition and analysis to understand the neural basis of the Mauthner initiated C-start escape of the goldfish. The initial biomechanical component, stage 1, is a sudden bending of the body whereas stage 2 is a forward acceleration that can also include a turn. Previously we reported results in which some of the kinematic parameters of the C-start were related to the underlying muscular activity based on EMG records. Here, we extend this analysis based on refinements in image and EMG processing. We report our findings based on seven strictly quantified kinematic descriptors that characterize the progression of the behavior.

Of the many correlations between the EMG and kinematic characters, of special interest is the fact that the rectified integral (volume) and duration of the stage 1 EMG burst are correlated with linear velocity and distance covered by the fish in stage 2. This suggests variable mono-neuron recruitment during stage 1. Moreover, the duration of stage 1 movement is proportional to the stage 2 EMG burst latency. From this we postulate that the command underlying the stage 2 contraction serves to terminate stage 1. If this is the case, then neurons triggering stage 2 are coded by the angle of the impinging stimulus. [Supported by NIH grant NS22621]

548.19


We are studying the Mauthner initiated C-start escape of the goldfish as a neuroethological model for sensorimotor coordination. This escape behavior has two biomechanical stages. Stage 1 is an initial bending of the body whereas stage 2 is a forward acceleration caused by a stroke of the tail. Stage 2 can also include a turn. To emulate predatory attacks, we dropped a ball into the water above the fish and we measured the relationship between the angle of attack and the stage 1 and 2 turns. We found that the attack angle reliably predicts these turns.

For stimuli behind the fish, stages 1 and 2 are small such that the animal accelerates forward in the direction of its initial orientation. To avoid stimuli in front, stages 1 and 2 are large so that the animal reverses its initial orientation. Thus, the direction of the stimulus can code the neural commands controlling stage 1 and 2 muscle contractions. In addition, nearby obstacles can modulate the command and alter the escape route. In cases when the fish made an error and turned toward the stimulus, there was no trajectory correction. Thus, once generated, the neural commands are resistant to subsequent sensory modulation even though such errors might increase the probability of capture. [Supported by NIH grant NS22621].

548.20

QUANTITATIVE AUTORADIOGRAPHIC STUDIES OF T3 AND IGF RECEPTOR BINDING AND PROTEIN SYNTHESIS IN THE BRAINS AND PINASE OF COHO SALMON DURING SMOLT TRANSFORMATION. Bern O. E. Ebbehusen*, Thomas Ostholm*, Dennis Basking*. Institute of Marine Science and Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775 and the Department of Medicine and Biological Structure, University of Washington, Seattle, WA 98108.

Smolt transformation (ST) occurs midlife in coho salmon. During this brief period coho salmon imprint on olfactory cues of their natal stream and change behavior. ST is associated with a plasma thyroid surge and our recent studies on the brains have revealed sequential changes in brain content of some monoamine and amino acid neurotransmitters, changes in immuno-reactivity of substances P and PAF-IMV in addition to extensive expansion of some axonal systems. In the search for factors controlling or affecting the neural systems associated with ST we have employed standard methods for quantitative autoradiography of T3 (in vivo) and IGF (in situ) receptor binding and methionine (35S) incorporation in salmon before, during and after ST. All three studies revealed significantly higher levels of radioactivity in the pineal than in any part of the brain. IGF receptor binding in the pineal and hypothalamus was high in the three salmon populations while the olfactory bulb in the smolt exhibited greater binding than in presmolts and postsmolts. The telencephalon in the smolt also showed greater binding than in pre- or postsmolts. Protein synthesis in the pineal, as measured by 35S labeled methionine incorporation, was exceptionally high in the three populations and in the olfactory bulb and telencephalon synthesis decreased in smolts and postsmolts. These results indicate that the pineal may play a significant role in ST and that the neural plasticity associated with it involves complex interactions of several hormonal and neurotransmitter systems.

Supported by research grants from NIH, Sea Grant and the V.A.
549.1

LEARNING SOCIETY of interaction from and feedback conspecific was of minutes, ERROR~DE SPEED section speed at 8ug ascending 549.5

MALIN, J.F. MUCK, B.J. NOVY, J.R. LAKE, R.E. PLUTCHER, A. LEHT HUNTO, J.W. NEEDHAM and D.J. GORDON, univ. of Houston-Clear Lake, Houston, TX 77580.

Galanin is a neuropeptide that coexists with aspartylcholine in the septohippocampal pathway. It appears to have a negative modulating influence on cholinergic transmission, suggesting that it might interfere with memory formation. The apparatus was a starburst maze consisting of a start box, and 5 radiating alley's: 4 level alley's and 1 baited ascending alley with a grid floor. The subjects were 20 Sprague-Dawley rats, handled for 10 days, cannulated in the body of the experiment with 7 punctures and divided to 0-9 of initial weight. Each rat was infused i.c.v. over 6 mins, with 8ug galanin in 24.1 saline or with saline alone. Twenty days after, conditions with infusions with MIF-1, each rat was placed in the maze and observed under "blind" conditions for number of errors (blind alleys entered) and latency to reach reward. Each rat was seen, latency was recorded. One day later, each rat was retested in the maze. Each rat's retention scores were its decrease in errors and increase in speed between the single training trial and the retention trial. Galanin-infused rats showed significantly less retention by both measures. (*) p<.05, ** p<.01 vs. saline.

M = SEM  
SALINE i.c.v. GALANIN Bug i.c.v.
ERROR DECREASE 3.1 ± 0.9 -1.1 ± 0.9 **  
SPEED INCREASE 0.7 ± 1.1

549.3


549.4 "Reproductive Memory" - A New Model for the Study of Recognition Processes in the Male Rat: Effect of Vasopressin Antagonist Administration. T. Mercieca-Wisniewski and V.D. Ramirez, Program as Neuroscience, University of Illinois, Urbana, Illinois, 61801.

Studies have shown that arginine vasopressin, AVP, modulates memory processing in the rat (De Wied, 1985, Prog. Brain Res, 60:135). Current work in our laboratory, using a novel model of recognition in the rat, has sought to assess the role of AVP in the rat's ability to recognize a familiar female conspecific. Sexually naive adult male rats were injected intraperitoneally with either 0.5 ml of saline or 0.5 ml of 1% saline containing 0.5 mg/ml of the AV antagonist, (7-O-Methyl-3, 8-Pseudopdynorphin), 2 cyclo[2-tyr]Arg]-AVP at a concentration of 10ng/ml, 10, 20, 30 minutes before testing. The female rats were paired with naive males and tested in the same manner as the males. It was observed that AVP antagonists prevented the males from demonstrating a preference for the novel female. This is similar to the results obtained in the human model of memory, where the AVP antagonist was shown to prevent the development of amnesia. The results of this study suggest that AVP may play a role in the development of memory in the rat. Further studies are needed to determine the exact role of AVP in the development of memory in the rat.
549.7 Vasoactive intestinal peptide (VIP): An amnesic neuropetide in mice. J.E. Morley, L.S. Oatley and J.E. Freud, Genetic Research, Educational and Clinical Center, VA Medical Center, and Division of Genomic Medicine, St. Louis University, St. Louis MO 63104

VIP is a neuropetide present in high concentrations in the hippocampus. Mice were prepared for intracerebroventricular (i.c.v.) or the hippocampal injections of VIP or saline 24 to 48 hours prior to training on a T-maze left-right footshock avoidance task. Importantly, injections of VIP or saline were administered i.c.v. (0 to 5.0 μg) or bilaterally to the rostral portion of the hippocampus (p.o. 1.0 μg total dose). When footshock avoidance training was continued without resulted in dose-dependent increases in the number of trials to make 5 avoidance responses in 6 consecutive trials by either route of administration. When a VIP receptor antagonist (1-G-D-Phe-Leu5-VIP) was administered into the hippocampus (0.25 μg), it yielded a dose-dependent improvement of retention, suggesting that VIP plays a physiological role in memory modulation.

The amnesic effect of VIP was not due to the side effects of any gastrointestinal, cardiovascular or respiratory actions of VIP. Neither VIP nor the antagonist antagonized the amnesic effect of VIP. The failure of VIP to block the amnesic effect of VIP was not due to the same route of administration for both neuropetides as centrally administered amnesics. This is concluded that VIP is a potent exogenous amnesic peptide.

549.8 GP120 and a VIP Receptor Antagonist Impair Morris Water Maze Performance in Rats. J.V. Pashino1, J.M. Hill2, D.E. Brennan3, M. Fridkin4, L. Groesch5 and J.R. Gloster6, Psychology Dept., The American University, Washington, DC 21209; 1Pepidie Design, Germantown, MD, 20874; 2LND, NICH, Bethesda, MD 20892; 3Weizmann Inst. Science, Rehovot, Israel; 4SCNE, NIMH, Bethesda, MD 20892

GP120, the protein coat of HIV, has been shown to be neurotropic to hippocampal cells in culture. These neurons support LTP, which is a considered a physiological model of learning. These findings may explain, in part, the impoverishment of memory seen in HIV positive patients. GP120 mediated neurotoxicity in culture can be prevented by co-treatment with VIP, suggesting a basis for therapeutic intervention. In order to develop a model of memory to assess the possibility, we attempted to create a comparable functional deficit in rats. We compared the effects of gp120 and a novel VIP receptor antagonist on the acquisition of Morris water maze performance. Male Sprague-Dawley rats were implanted with i.c.v. cannuli and dosed (once daily) with different agents for 7 days before exposure to the maze, and (1 hr before testing) for the entire training period. In control (saline and unoperated) rats, performance progressively improved over 15 days of training. GP120 and the VIP antagonist impaired the acquisition of this performance. This results strongly suggest that experimental blockade of VIP receptors, or treatment with gp120, can impair performance in a learning- and memory-related task.

549.9 Effect of monoaminergic drugs on memory in mice. R.F. Rittmann, A.Kling, A.Glasky, K.Lees, A.Gevorkyan*

R.F. Rittmann and A. Gevorkyan*

Advanced Immunotheapeutics, VAMC/UCLA Sepulveda CA 91343

An animal model has been proposed for human memory loss which occurs during aging. This model is based on the observation that a T-maze once a rat enters a goal box and consumes all the food, on the next trial it will enter the other goal box. By increasing the time between trial it can be determined if the rat can remember which side of the maze it entered for the previous trial. While this model has been well documented in rats it has not been tested in mice. In the present study male Swiss Webster mice, food deprived for 90% of their normal caloric intake were tested in this model. At delays of 30 or 60 seconds a correct response occurred 75% of the time. When the delay was increased to 90 seconds, the correct response rate fell to chance (46%). Since there is a considerable amount of interest in comparing immune and brain function we tested two compounds with immunomodulatory activity, AIT0082 and AIT0083, in this model. At low doses (0.5 mg/kg) both compounds improved performance at the 90 second delay to 65-68% correct. At high doses (30 mg/kg) AIT0082 improved performance to 80% correct, but AIT0083 treated mice performed at chance level. Further studies indicated that AIT0083 at the high dose reduced performance at both the 30 and 60 second delays to chance while saline injected mice were correct 75% of the time. Supported by VANC Research Service a grant from AIT.

549.10 Amygdala injections of dizapam facilitate long-term decrements of the acoustic startle response in rats by reducing fear/sensitization. B.J. Young, S.A. Rabiei and N.R. Scharfman, Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Borsook, Cranney, and Leaton (1989) showed that fear conditioning in an acoustic startle habituation paradigm may mask long-term habituation through a long-term sensitization process. Physiological and behavioral manipulations that reduced fear, as indexed by freezing behavior, increased the rate of habituation. We tested the effects of injections of the anxiolytic drug, diazepam (Young, Helmstetter, and Leaton, 1988), facilitated long-term decrements of the acoustic startle response. The amygdala is known to mediate the anxiety state (Nagy, Zamo, and Doci, 1979), and lesions of the amygdala, like systemic dizepam, facilitated long-term acoustic response decrements (Leaton and Supple, 1987). Therefore, injections of dizapam into the amygdala should facilitate long-term response decrements. Eight rats received bilateral intra-amygdaloid injections of diazepam (55 μg in 1 μl) prior to habituation training in the acoustic startle chamber, and 8 rats served as vehicle-injected controls. Ten stimuli were presented on a 60-sec interstimulus interval every other day for a total of three sessions. As predicted, diazepam injections (1) significantly enhanced, long-term decrements of the acoustic startle response, (2) did not alter initial response levels, and (3) reduced freezing in the startle chamber during the early stages of training. We conclude that the facilitatory effect of diazepam on long-term decrements of the acoustic startle response is related to its fear/sensitization reducing effects, and these effects may be mediated through the amygdala.

549.11 Anxiolytic and anxiogenic drugs on the early acquisition of two-way shuttle avoidance in rats. A. Fernández Temuel, R.M. Escorihuela*, A. Zagoza, J.J.M. Núñez*, E. Boix*, A. Júnquera* and J. González Medical Psychology Unit, Dept. of Pharmacology & Psychiatry, School of Medicine, Autonomous University of Barcelona, 08025-Bellaterra, Barcelona-SPAIN

Genetic evidence suggests that anxiety-like posttraumatic or adult re-activation to handling, had shown that the early acquisition of the two-way shuttle avoidance is an anxiety-mediating behavior. In the present study, we test psychological evidence to this view by systematically testing the action of anxiolytic and anxiogenic drugs on that task. In 25 trials with mild shock (0.4 ma-0.6 ma) were used. In the first experiments the action of sodium pentobarbital (1.25, 2.5 and 5 mg/kg), and three benzodiazepine drugs, diazepam, alprazolam (1, 1.25 and 1.5 mg/kg) and adrafinil (1, 2 and 6 mg/kg) were tested. The last two experiments were carried out to test a possible facilitation or sensitization action of sodium pentobarbital (0.5, 1 and 2 mg/kg) and FG 7145 (5, 10 and 15 mg/kg). Two partial inverse agonists of benzodiazepine receptors, preceded with previous data on a model with different model and men. The results showed that the measure of early acquisition of two-way active avoidance is sensitive to the action of anxiolytic or anxiogenic effects of drugs.


Diazepam (Valium®), a frequently prescribed benzodiazepine, impairs acquisition, but not retention of spatial information (Neugebauer, Biobehav. Rev., 9:87, 1985). Diazepam also impairs place learning in the Morris Water Maze (McNamara & Whisnant, Psychopharmacology in press). This study sought to be the impairment of spatial learning.

Three groups of rats were trained (14 days, 4 trials/day) to locate a hidden platform maintained in a submerged opaque water. All groups were injected IP 20 min before training with either saline or diazepam (3 mg/kg). The diazepam and saline groups received those injections throughout training but the saline (saline-diazepam) was switched from saline to diazepam after reaching criterion performance on the seventh day of testing.saline-diazepam was switched from saline to diazepam after reaching criterion performance on the seventh day of testing. Relative to the saline group, the diazepam group showed longer path, greater heading errors, and slower swim speeds. The saline-diazepam switch group showed slower lower under diazepam but showed no other deficit in maze performance. When the platform was removed from the pool, the saline and saline-diazepam groups, but not the diazepam group, showed a preference for the quadrant in which the platform was located. This finding could be attributed to the manipulation of diazepam in the maze when the platform was located in a different quadrant than the platform was located in the pool. These data suggest that diazepam impairs the formation of new spatial memories but not the utilization of previously stored spatial information. (This research was supported by an NSERC operating grant awarded to RWS, and UVic. fellowship to RKM).
549.13 CHRONIC FLUMAZENIL ENHANCES LEARNING OF A SWIM-ESCAPE TASK IN RATS. M. Urbancic and T.J. Marczynski, Dept. of Pharmacology, Univ. of Illinois, College of Med., Chicago, IL 60612.

Chronic administration of the benzodiazepine receptor antagonist flumazenil, was previously shown to have an anxiolytic effect in the elevated plus-maze test and the punished-drinking test (Urbancic, M. et al. Pharmacol. Biochem. & Behav., 35:505-509, 1990). Since the memory-enhancing effect of flumazenil was proposed (Lal, H. et al. FASEB J.), we investigated the mnemonic effects of this drug during chronic administration. Rats pretreated with flumazenil (4 mg/kg/day in the drinking water for 2 or 3 weeks) were tested for acquisition and retention of a swim-escape response in the round water tank and in the water T-maze. Flumazenil-treated group required fewer trials to reach the acquisition criterion than controls. When the rats were tested for retention 3 days after the last trial (without further drug treatment) there was no difference between the groups, but when tested on day 5 after drug withdrawal, the control group had greater difficulty reversing the maze habit than the flumazenil-exposed rats. These findings indicate that chronic flumazenil seems to combine anxiolytic action with enhancement of learning, in contrast to benzodiazepine antagonists whose anxiolytic action is accompanied with impairment of learning and memory. Supported by USAF grant 87-0364.

549.15 REVERSAL OF DIAZEPAM-INDUCED IMPAIRMENT IN DISCRIMINATION PERFORMANCE BY RO 15-1788, S.-O. CONLON, Department of Psychology, Rutgers University, Camden, NJ 08102.

The effects of diazepam (DZ) alone and in combination with Ro 15-1788 on the performance of a previously-learned go-no go successive discrimination were studied in male, Sprague-Dawley rats. DZ 4 mg/kg impaired discrimination performance in five of six sessions, although animals showed some tolerance to the drug's action. The impairment in discrimination performance was due to an increase in responding during no go periods of the task (effects of commission). The benzodiazepine (BZ) receptor antagonist Ro 15-1788 (5 and 10 mg/kg) reversed the impairment in discrimination performance and reduced the number of incorrect responses in a generally dose-dependent manner when co-administered with DZ. These findings suggest that the impairment in discrimination performance by DZ is mediated by central BZ receptor sites. When administered alone, Ro 15-1788 10 mg/kg (but not 5 mg/kg) produced a mild impairment in discrimination performance. However, in contrast to the effects of DZ, this impairment was due to both small increases in no go period responses (errors of commission) and small decreases in go period responses (errors of omission). These findings suggest that Ro 15-1788 is not a neutral antagonist but has some intrinsic action of its own.

549.14 ANXIOLYTIC CENTRAL AND PERIPHERAL BENZODIZEPINE RECEPTOR LIGANDS EIGHT DIFFERENT EFFECTS IN LEARNING AND MEMORY TESTS IN RATS. P.V. Holmes & R.C. Drugan. Schair Research Lab., Dept. of Psychology, BCCM University, Providence, RI 02912.

Previous research has demonstrated that low doses of anxiolytic central benzodiazepine receptor (CBR) ligands, the beta-carbolines, improve performance in various learning and memory tests in animals if administered prior to training. The present experiments compared the effect of a beta-carboline (PG 7142) with that of a pharmacologically distinct, anxiogenic peripheral ligand, the benzodiazepine receptor (PBR) ligand (NS-4864), in two tests of learning and memory in rats. As expected, PG 7142 significantly improved performance in a passive avoidance test, NS-4864 was without effect. In a shuttlebox escape test, NS-4864 significantly impaired performance while PG 7142 had no effect. The effect of NS-4864 was antagonized by the specific peripheral benzodiazepine receptor antagonist, PR-1193. These results indicate that the differential impact of CBR and PBR anxiogenic ligands on performance in averstively-motivated learning tests may be a reflection of their distinct pharmacology. Furthermore, the anxiolytic enhancement of drug does not appear to be a sufficient requirement for enhancing acquisition in averstively-motivated learning. Supported by NIMH grant MH44034-02A1 & an Alfred P. Sloan Research Fellowship, #R8-8952 to Robert C. Drugan.

549.16 AN ELECTROPHYSIOLOGICAL COMPARISON OF MOD 26,479, A NOVEL TRIPTAZOLE, AND THE BETACAROLINE SLICE: EVIDENCE FOR COGNITION ENHANCING POTENTIAL. S.N. Sorenson, J.M. Zvolensky* and T.M. Humphreys*, Merrell Dow Research Institute, Cincinnati, OH 45256.

Compounds which enhance memory in behavioral models have been shown to augment long term potentiation (LTP) in the hippocampus. Many of these compounds also have effects on the basal population spike amplitude. The beta-carbolines with benzodiazepine inverse agonist activity have been shown to enhance learning in animals. In these experiments we compared the effects of two beta-carbolines, DMMC and beta-CDM, with those of MOD 26,479, a novel triazol which may have cognition enhancing potential, in the hippocampus. DMMC (5 µM) and beta-CDM (10 µM), increased the basal population spike amplitude of CA1 pyramidal neurons and the magnitude of the LTP produced by a tetanizing stimulus. At higher concentrations however, these compounds produced epileptiform activity in the hippocampal slice, consistent with their high dose convulsant properties. MOD 26,479 (20 µM) produced similar effects on the basal population spike amplitude and on LTP suggesting that this compound will also have memory enhancing potential. Unlike the beta-carbolines however, high doses of MOD 26,479 did not produce epileptiform activity, consistent with behavioral evidence that this compound does not have the high dose convulsant liability seen with many beta-carbolines.

549.17 COGNITIVE DEFICITS IN 16MO F-344 RATS AND IMPROVED PERFORMANCE WITH BMY 21502. H.D. Lindner, S.L. Moon,* and V.K. Grishkoff, Bristol-Myers Squibb, CNB Biology, P.O. Box 5100, Wallingford, CT 06492-7600.

Morris Water Maze testing with a 60 minute inter-trial interval and 2 trials per day for 5 days revealed that 16mo F-344 male rats were dramatically impaired relative to 2 mo rats in acquiring this spatial-memory task as measured by the swim distance to locate a submerged stationary platform. Impaired visual acuity was noted in 16mo rats by longer swim distances to a platform marked with a small visible cue than 2mo rats. 16mo rats were also vulnerable to hypothermia. Surprisingly, 16mo rats were already 0.5°C colder than 2mo rats at baseline and their rectal temperatures dropped 0.5°C from baseline after a 60sec swim while 2mo rats maintained their temperatures. The possibility that these non-cognitive factors, poor visual acuity and hypothermia, might completely account for the deficits in spatial mapping performance was not supported by analysis of covariance or stepwise multiple regression. Age alone was the best predictor of spatial mapping ability which suggests that the deficit in 16mo rats may be partially due to some higher cognitive deficiency. In contrast, the older rats' spatial mapping deficit was manifest as a delay in the appearance of a trials effect (improvement from trial one to trial two). BMY 21502, which has been shown to prolong LTP (Grishkoff, Neuropsychopharmacology, 1990, in press) accelerated the appearance and magnitude of the trials effect in 16 mo rats.

549.18 PIRACETAM AND BMY 21502 FACILITATE PERFORMANCE OF TWO-CHOICE MIDDLE-WATER-ESCAPE IN NORMAL RATS. N.M. Mean*, T.R. Corner*, and R. Moore*, Department of Psychology, East Carolina University, Greenville, NC 27858.

Sprague-Dawley rats given either 5 or 10 mg/kg BMY 21502, 150 mg/kg piracetam or methylcellulose vehicle (p.o.) daily for 38 days beginning two days before intracranial self-competition on performance of a win-stay/lose-escape task that required the use of trial-dependent working memory. Testing was conducted in a circular water maze that was divided into a start section and two choice sections by a T-shaped metal barrier. The task involved giving the rats pairs of trials in which the spatial location of a submerged escape platform remained in the same choice section within a pair of trials but changed semi-randomly across pairs. Rates receiving either 5 or 10 mg/kg BMY 21502 or piracetam made more correct choices than did rats receiving only the vehicle (p<0.05 in each case). The facilitated performance was associated with making fewer perseverative responses that resulted in errors.
550.1

Microspectrophotometry (MSP) has shown that R1-6 receptors in white-eyed Drosophila's compound eye have a daily visual pigment rhythm (decreasing to 60% of its height after light onset, then recovering an hour equivalently). In trying to relate pigment turnover, membrane cycling, and sensitivity, we followed our MSP with electron microscopy (EM), morphometry, and electrophysiology (electroretinography, ERG). EM revealed that the cytoarchitecture of membrane cycling (autophagy and renewal) in white-eyed per* controls remained constant throughout a 12 ± 18 hours cycle. Morphometry showed that there were no significant changes in rhabdome cross sectional area in this diel photoperiod. However, the ERG corroborated the L:D and D:D cycles of visual pigment, with these details: (1) sensitivity varied about twice as much as visual pigment; (2) sensitivity decreases preceded visual pigment decreases by several hours; (3) UV and blue sensitivities varied in parallel in L:D but UV sensitivity decreased preferentially in D:D; (4) per mutants (L = long and s = short) had little influence on the timing of the ERG cycle in D:D; (5) per has lower and lower sensitivity. Supported by NSF grant BNS 88 11062 and NIH grant ROI EY 07192 to WSS.

550.2
CIRCADIAN PACEMAKER CONTROL OF LOCOMOTOR ACTIVITY PHASE IN BULLA GOLDFULVA. M. R. Roberts and X. Xin.* Department of Biology, Clarkson University, Potsdam, NY, 13699-5805.

In order to determine whether the circadian pacemakers of the marine snail Bulla goldfulva, exert phase control over the circadian rhythm in locomotor activity, we measured the phase angle for entrainment of the circadian pacemakers and the activity rhythm on four different photoperiods: L:D 15:9, 12:12, 9:15, and 4:20. We found that the phase angle for ocular entrainment was progressively advanced relative to dawn as photoperiod decreased (0.31±0.04 [29], 1.85±1.36 [10], 3.00±0.74 [22], 4.92±2.30 [17]); hence, although the phase was fixed relative to the middle of the day. This entrained phase match predictions based upon the two-pulse non-parametric entrainment model of Pittendrigh. In contrast, activity began near dusk on all photoperiods. On subsequent release into constant conditions, the free-running locomotor activity rhythm commenced near the time of previous activity onset. Thus, activity phase on light cycles represents the entrainment of a light sensitive pacemaker. The resulting liability in phase between ocular and behavioral rhythms in Bulla exposed to light cycles suggests that the ocular pacemakers are not the only determinants of locomotor activity phase in Bulla. We conclude that the circadian eyes of Bulla goldfulva consists of several pacemakers, some outside the eyes, whose internal phase relationship can be modified by altering photoperiod. Supported by NS26172 to MSF

550.3
THE ROLE OF CHLORIDE CONDUCTANCE IN THE PERIOD SHORTENING OF THE CIRCADIAN PACEMAKER IN BULLA S. Michél, S.B.S. Khalsa & G.D. Block Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901.

The eyes of the marine snail Bulla express a circadian rhythm of compound action potentials (CAPs) in vitro. Although hyperpolarizing and depolarizing chronotropic treatments both lengthen the period of the pacemaker inhibition of Ct- conductance has been recently found to shorten the period by up to 2.5 hr. In order to address the possible mechanism underlying this period shortening we examined the phase effect of Ct- conductance. Detailed analysis of the CAP rhythms in Ct- free artificial seawater (ASW, Ct- substituted with SO42-) showed a significant decrease in the length of the non-stationary period of the eye. By the late subjective night, suggesting the involvement of Ct- conductance during this phase of the cycle. The phase response curve for 6 hour pulses of Ct- free ASW shows small phase advances during the subjective day (CT 5:11; min 16, CT 8:14; 35 min ±15) and larger advances during the subjective night (CT 14:20; +47 min ±9, CT 17:25; 95 min ±14). The observed phase advances during the late subjective night (CT 7:23) are comparable to those previously obtained with depolarization, whereas those during the late subjective day (CT 8:14) are comparable to phase shifts to hyperpolarization. Thus the advance phase response curve could be a result of an alternation of the circadian rhythm in membrane potential response to a Ct- conductance inhibition. Ct- conductance could act as a passive leak damping the circadian membrane oscillations, or, alternatively, be actively driven by the pacemaker. Intracellular recordings currently underway should help identify the role of Ct- conductance in setting the circadian period. Supported by DFG Mo. 1281-1 to SM, NRSN 509621 to SBSS and NS15204 to GDB.

550.4
THE SPIKING PHOTORECEPTORS OF THE BULLA RETINA. M.E. Geuze and G.D. Block, Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

When the eye of the mollusk Bulla goldfulva is maintained in constant darkness at 15°C, two circadian rhythms occur in the frequency of impulses in the optic nerve. One rhythm, with a period of about 24 hours, is a compound action potential frequency, is believed to be generated by neurons of the basal retina (Block et al., 1984). The second, a rhythm in small impulse frequency (Geuze and Page, 1990), could be generated by spiking photoreceptors of the distal retina. Electrophysiological recordings made extracellularly from the spiking photoreceptors occur in synchrony with small spikes in the optic nerve, whether they are generated spontaneously, through current injection, or by light. Impulses from the spiking photoreceptors cease briefly following a light onset, and then resume firing at an increased rate. The spiking photoreceptors display characteristics of the previously described H-type cells of Bulla, and may account for the firing pattern of small impulses in the optic nerve in darkness. Supported by NS08806 to MEG and NS15264 to GDB.

550.5
CHARACTERIZATION OF A PUTATIVE CIRCADIAN OSCILLATOR PROTEIN IN THE EYE OF Aplysia. L. Rains, M. Nager-Reguero*, B. Cook*, and A. Fishkin, Dept. of Biochemistry, Univ. of Houston, Houston College of Medicine, Houston, TX 77030.

Elucidation of the circadian oscillating mechanism entails identifying its components, determining how the components interact with one another and testing whether these interactions account for properties of the circadian rhythm. A reasonable hypothesis is that proteins are components of entrainment pathways and they also are components of the oscillator. Light and serotonin (5-HT) both regulate the circadian rhythms in sheep. Therefore, we have looked for changes in proteins whose expression are modified by light or 5-HT. Using 3-D gel electrophoresis to separate proteins, we found that exposure of eyes to light or 5-HT altered the incorporation of amino acids into membrane proteins. Immunoblot analysis of proteins whose expression were modified by both light and 5-HT, a number of proteins may be considered putative components of the oscillator. As a first step towards determining the role of these proteins, we are obtaining amino acid sequences of the proteins. Protocols were cut from 2-D gels, digested with V8 protease, and then separated on a 1-D gel. Peptides were excised into phospholipid-membrane and then incorporated into a gas-phase sequencer. A 38 amino acid sequence of a peptide was obtained from a protein (~40k, pl 5.6) that was affected in opposite ways by light and 5-HT. Most exciting and important was the discovery of a significant (~150–300) changes in our sequence and published sequences of a family of proteins called Tocipores. This family of calcium and phospholipid binding proteins is believed to play important roles in cellular regulation. The tentative identification of the 40k as a Tocipore has given us new ideas about where to look for the circadian oscillating mechanism. More specific hypotheses can now be tested based on the possible cellular functions of this protein.

550.6

One widespread effect of constant white light applied on electrophototogenic (EOP) rhythms in crayfish is changing in the period (ΔT) of the freerunning rhythm. In previous works, we observe that it is possible that this change is driven by other factors as well. In order to analyze a possible change on angular velocity ERG when monochromatic light is applied in continuous way. Adult and intact crayfish Procambarus clarkii were monochromatic acute white light adapted (Δ 465, 555 and 630 nm) with a 10 lux intensity during six days. The ERG was recorded by means of a metal electrode into the cornea to lead the photoreceptor’s electrical response. The change in period (ΔT) length depends on the λ employed, thus, the lag between the EOP acrophase obtained with white light and with blue light was 210°, with green light (565 nm) was 20°, and with red light (630 nm) was 180°. These results indicated that the change in angular velocity caused the observed change which depends on one photoreceptor’s group altered by the λ used.

The electrotetrogram (ERG) in the crayfish shows during the early stages of ontogeny a clear circadian rhythm (24 h) which antedates the circadian rhythm (CR). On the other hand, a persistent ERG amplitude CR with superimposed ultradian oscillations has been established in the isolated eyestalk of the adult crayfish. These findings suggest that the UR represent the output of a multioscillator system which, when in phase, gives rise to the overt CR. The goal of this work was to analyze the mechanism of temperature compensation in both the juvenile stages and the excited eyestalk of the adult in order to prove that the UR and CR share this property. ERG recordings were performed individually in both preparations under darkness for at least six days; the recording period was 90 min. The study was conducted in a temperature range of 18°C to 31°C and the results are reported separately.


The protocerebrum of crayfish contains a pair of bilateral homologous circadian pacemakers driving retinal sensitivity (B. Barrera-Mera and S. Socrosci, Abstr. 11, 1985) and glucose concentration in hemolymph (B. Barrera-Mera and S. Móth-Mánt, Soc. Neurosci. Abstr., 1979). For this reason, we have determined the circadian locomotion pattern in crayfish maintained in different conditions of light and darkness. We will report the results obtained in different conditions of light and darkness. We propose that the locomotor activity pattern in crayfish is due to the interaction between two populations of circadian pacemakers which are coordinated by a central nervous system.
CIRCULATORY RESPONSES of MANDUCA SEKTA FLIGHT. H.K. Lehman, ARL- Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

Circadian clocks have been described in a wide variety of organisms and are known to control several physiological processes, yet the neurotransmitters that mediate these processes are less well known. I have focused on the circadian flight of the insect, Manduca sexta, to study transmitters and pathways in which a circadian clock controls behavior.

Flight activity occurred during a diurnal flight schedule of individually restrained moths. Flight activity persisted in constant darkness, had a period length slightly less than 24 hours, and could be phase shifted. Circadian rhythms were monitored in the brain using chronobiological techniques, in the hemolymph from <10 to 95 pmoles/90 ul. The greatest concentrations in each time course were detected at 1-4 PM and 4 AM, coincident with peak levels of flight activity. In addition, injections of the octopamine agonist, chlorphostrin, produced long lasting flight activity. Recently, immunocytochemical techniques have been used to visualize octopaminergic neurons in the brain, and studies are underway to characterize these neurons in order to determine their role in the circadian control of flight.

Supported by grants to J. G. Hildebrand.


To investigate the genetic determinants of the effects of light on the circadian rhythms in mammals, male mice of several inbred strains were monitored under constant light of successively increasing intensities, individually, and their locomotor activity was monitored over the course of several months using photoelectric beams. The C57Bl/6J mice seemed to have an all-or-nothing response to light. Their period ( ) increased with a change from constant dark to 8 lux constant light but did not increase further when the light intensity was increased to 93 lux. Furthermore, the daily bimodal pattern of locomotor activity of the C57 mice was lost when going from light: dark or dark: dark conditions to constant light. I can contrast the period of activity for the DBA/2J mice increased as the light intensity increased from 0 to 8 lux and increased again as the light intensity went to 93 lux.

550.16 CIRCADIAN RHYTHMICITY IN SHR AND WKY RATS: EFFECTS OF LIGHT INTENSITY. A.M. Rosenwasser, Dept. of Psychol., Univ. of Maine, Orono, ME 04078.

Recent studies from this laboratory suggest that a noradrenergic mechanism may interact with light intensity to alter free-running circadian rhythm. In the course of these studies, it was noted that the spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats, known to display characteristic behavioral and neurochemical differences, also differed in free-running period under constant light. In the present study, SHR and WKY rats were monitored under a series of increasing light intensities, beginning with constant darkness. Least-squares spectral analysis was used to quantify the parameters of free-running rhythms. As expected, periods lengthened, amplitudes decreased, and spectral profiles became more complex, with increasing light intensity. Strain differences in period, characterized by longer periods in the WKY strain, were seen only under the highest intensities, while strain differences in amplitude, characterized by greater amplitudes in the WKY strain, were seen only under the lowest intensities. In contrast, the two strains showed similar spectral profiles, and appeared equally likely to show disrupted rhythmicity under bright light. The strain by light intensity interactions seen in this study may be due to strain differences in noradrenergic systems.

550.17 RESPONSES OF MONTANE VOLES TO INCREMENTAL PHOTOPERIODS. C.N. Rosemmiti and P.J. Berger*, Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

Previous work suggests that montane voles may respond to small changes in day length at the winter solstice (WS) to schedule changes in body weight (Petterborg, L.J. Can. J. Zool. 55:431, 1977). We housed adult male voles housed under LD 16:8 under decreasing photoperiod in the laboratory (~2 min/day) from Oct. 18 until WS at which time animals were divided into 3 groups: A-natural light (~2 min/day), and C-solstice hold (LD 9:54:14:06). Controls were housed under LD 12:12 (Group D) or LD 16:8 (Group C) after Oct. 18. Group A was exposed to the decreasing light in an environment with natural lighting. Animals were sacrificed on Feb. 21. Groups A, C, and D lost weight between mid-Nov. and WS and maintained their weights for the remaining month. Group B lost weight between mid-Nov. and WS but gained weight in the following month. Group E gained weight in both groups during this interval. Analysis of covariance of log tested weight with log body weight as covariate demonstrates a significant effect of treatment. Group E has higher reproductive function than group B, with a trend existing when groups A and C are compared. Thus, post-solstice changes in growth rates appear to be spontaneous. However, gonadal function may respond to incremental changes in photoperiod.


The visual sensitivity of the hamster circadian pacemaker to brief stimuli has been studied extensively. Using the phase-shifting response to brief light pulses as an assay, we have shown that this pacemaker is not an integrator of the total photons in a single stimulus but does not light-adapt to ambient illumination. We have measured the photic sensitivity of the freerunning period of the circadian rhythm to continuous illumination levels that vary 6 log units. Golden hamsters were maintained in constant light (23 ± 0.3 lux) and 100% light of 240 lux with 100% light of 240 lux with 0.5 lux. The freerunning period was measured after 1, 6, and 9 weeks. One group remained in darkness as a control. During week 1 of constant conditions the period of the activity rhythm was shortened monotonically with increasing illumination. At light levels less than 10^3 lux light 4^3 lux the freerunning period was not different from the period measured in constant light. Between 10^3 and 10^4 lux the freerunning period was shortened (23.70 ± 0.03 h at 6 x 10^4 lux light 4^3 lux). After 6 weeks in constant conditions a biphasic relationship had developed between the constant illumination and the freerunning period. The freerunning period of the hamsters in low and high levels of illumination were similar (approximately 24 h) by the periods of the animals kept in intermediate levels of illumination (approximately 23 h) were significantly shortened (23.20 ± 0.06 h). After 9 weeks in constant conditions the period of the rhythm for hamsters in intermediate levels of constant light remained significantly shorter (23 ± 0.02 h) than the periods of both animals in darkness (24 ± 0.02 h) and the highest levels of illumination (24 ± 0.02 h). The shorter freerunning period in hamsters maintained in intermediate levels (23 ± 0.02 h) of constant light has never been quantified however it has been predicted by Duan and Pittendrigh (J. Comp. Physiol. 106:197, 1979) based upon the hamster’s response to intermediate levels of light. Our results suggest that changes in the freerunning period of the hamster circadian pacemaker to light can be predicted by the sensitivity of the pacemaker to brief light pulses.
EPILEPSY: ANIMAL MODELS

551.1 NAPPING WOLFFE BRAIN ACTIVITY DURING DRUG INDUCED SEIZURES WITH VOLTAGE SENSITIVE DYES. F.J. Salas, and W.R. Barbour, Dept. of Veterinary Affairs, VMC, Univ. of Pittsburgh, PA 15261, and Pittsburgh Epilepsy Center.

The functional interrelationship between electrical activity and brain function is a central concern in the study of epilepsy. In vivo mapping of nerve pathways during seizures can uncover new structure-function relationships. It is demonstrated that the voltage sensitive dye D10-31H-5 is a fluorescent marker of electrical activity in neural structures during drug induced seizures in awake animals. The technique allows a complete histological survey of the brain (exp. neural 105:165-185, 1989). The rats (yoked from surgery to image analysis) received one of four treatments prior to dye injection: 1) Intracerebral injection of 2,25 mg/kg of bicuculline (BIC) + clonidine (CL) or 2) Intracerebral injection of 1000 ml saline, 3) Injection of 12 mg/kg of kainic acid (KA) + saline vehicle, or 4) Injection of saline alone. EEG activity was recorded via hippocampal electrodes and the dye injection site was confirmed by iron staining. The activity recorded was the neuronal activity of the septum, amygdala, anterior thalamus, hippocampus, globular corpora, substantia nigra, and entorhinal, frontal and posterior cortices was mapped.

Past work has shown that different patterns of hippocampal hypoperfusion and depolarization occurred during seizures induced by KA and BIC. This suggests that the spatiotemporal pattern of polarization throughout the brain would be characteristic of different seizure inducing agents. These patterns should provide insight into both mechanisms of seizure propagation and strategies for medical interventions.

551.2 ALPHA-2 ADRENERGIC CONTROL OF THALAMIC OSCILLATION. G. Burzsky, C. Slomka*, A. Suljani*, F. H. Gage, E. Rossboli*, and M. Hsu, Dept. of Neurosciences, UCSD, La Jolla, CA 92039.

The effects of alpha-2adrenergic drugs on neocortical high voltage spindle discharges (HVS), reflecting thalamic oscillation, were investigated in freely moving rats. Bilateral microinjections of the alpha-2 agonist, xylazine, and clonidine into the n. ventralis lateralis areas bilaterally of the rat abolished the hippocampus or corpus callousum, as powerfully increased the incidence and duration of HVS as when these drugs were given peripherally. The HVS-promoting effect of clonidine was antagonized by prior intrahippocampal injection of the alpha-2 antagonist, yohimbine. The amplitude of the HVS was increased by picomole amounts of unilaterally-injected clonidine. Neurotoxic destruction of the thalamic noradrenergic afferents by prior intrahippocampal injection of 6-OHDA increased the incidence of HVS. Importantly, intrahippocampal administration of xylazine continued to induce HVS even after destroying the thalamic noradrenergic terminals. Downregulation of alpha-2 receptors by amitriptiline reduced the effectiveness of xylazine. We suggest that a major action of alpha-2 adrenergic drugs on thalamic oscillation is mediated by postsynaptic alpha-2 adrenergic receptors located on the thalamocortical neurons and that the final physiological action of norpamine on thalamic oscillation is a function of the relative density and affinity of alpha-1 and alpha-2 adrenergic subtypes.
EPILEPSY: ANIMAL MODELS

551.3 CHRONIC AND ACUTE EFFECTS OF TETANUS TOXIN IN RAT NEOCORTEX. Y. Chagnac-Amatii, K. Brenner* and N.J. Outtsick, Dept. of Physiology, Ben-Gurion University of the Negev, Beersheba, Israel.

We previously showed that injection of 50 MLDS of tetanus toxin (TT) into rat neocortex produces a potent chronic epileptic focus (J. Physiol. 37:364, 1989). We now describe the neuronal activities in neocortical slices prepared from both hemispheres of rats that had been unilaterally injected with a minute quantity of TT (2-10 MLDS in 0.5 μl) 16 hours to 35 weeks earlier. All slices showed hypersynchronous activity; spontaneous discharges were observed in 50% of the experiments. These took the form of prolonged, negative field potentials which coincided with intracellular Ds. In most slices, there was evidence of residual postsynaptic inhibition, despite the profound epileptogenesis. However, IPSPs were only recorded in restricted regions of a given slice. APV (20 μM) abolished most of the DS, often unmasking a late, prolonged hyperpolarization. This is in contrast with the moderate effect of NMDA blockers on focal epileptogenesis induced by acute convulsants.

Hypersynchronous activity also followed exposure of normal tissue to TT. This effect was dose and time dependent. Although fast IPSPs were selectively reduced at first, prolonged inhibition led to eventual blockade of all synaptic transmission.

Supported by the DFG (SFB 200).


Although most seizures are thought to be mediated by hippocampus, under certain circumstances the spinal cord can play an important role. We used frogs with thoracic spinal cord transections to investigate this question for several chemical epileptogens. Strychine induced status epilepticus with tonic-clonic convulsions and then seizures were identical in the intact or cord-transected animal. This is consistent with the seizures originating in the spinal cord. Cls-platin (cis-di(aminedichloro-platinum II) caused tonic-clonic seizures in intact frogs; after cord transection, seizure activity occurred in the upper extremity only while the lower extremity remained flaccid. These observations suggest that the seizures originated in structures above the level of the cord. Kainic and pentylene tetrozole gave intermediate results. With these agents, cord transected frogs showed some activity of the lower extremities (but decreased from that seen in the upper extremities), suggesting that spinal cord activity may contribute to seizures.


γ-Hydroxybutyric acid (GHB), a naturally occurring metabolite of GABA, induces bilaterally synchronous spike wave discharges (SWD) associated with behavioral changes reminiscent of petit mal seizures when given to animals. The GHB-treated animal thus represents a useful experimental model of generalized absence seizures. Although there is a wealth of data concerning excitatory amino acid (EAA) mechanisms in experimental generalized convulsive seizures, there is little information concerning EAA in animal models of absence seizures. In the present study, the effect of the noncompetitive antagionate of the NMDA receptor, MK-801, on GHB-induced SWD was assessed using dose response and time course studies in rats chronically implanted with cortical electrodes which allowed continuous EEG recording in the freely moving state during all phases of the experimental protocol. The GHB model of absence seizures was standardized and quantified as previously described (Snedd, Epilepsia 29:361, 1988).

The dose range studied was 0.1-1 mg/kg. MK-801 was used at 0.1 mg/kg. MK-801 had a complex biphasic effect on the GHB-induced seizure in that it produced significant prolongation of the SWD in the early stages of seizure (25% increase), but attenuated later stages of the absence-episode. Lower doses (0.1 mg/kg) of MK-801 resulted in a 35% delay in latency to onset of SWD while doses of MK-801 in excess of 1 mg/kg resulted in a 100% increase in the mean duration of absence seizures.

These data raise the possibility that EAA-mediated mechanisms may be involved in the genesis of GHB-induced SWD in this experimental model of generalized absence seizures.


γ-Hydroxybutyric acid is a naturally occurring compound which has the ability to produce spike wave discharges (SWD) associated with behavioral arrest. The GHB-treated animal thus represents a useful experimental model of absence seizures. Both clinical and experimental absence seizures, including the SWD model, are surmounted by GABAergic agonists. The purpose of this work was to explore possible GABAergic mechanisms in GHB-induced SWD.

The status of the GABA/ACh receptor/ionophore was determined during GHB-induced SWD and also in the presence of GHB in varying concentrations in vitro experiments. GHB had no effect on the binding of [3H]-bicuculline, its allosteric modulation by GABA, or [3H]-TPS-binding, but did result in a 15-20% decrease in [3H]-furinazepam binding with no effect on the allosteroic modulation of this site by GABA.

At the onset of the SWD induced by GHB there was a significant decrease of [3H]-furinazepam binding in brain which continued for the duration of the SWD. There was no change of [3H]-muscarinic binding at the onset of SWD, but one min after SWD onset, the was a significant, albeit transient, increase of [3H]-muscarinic binding. Allosteric modulation of [3H]-muscarinic and [3H]-furinazepam binding was unchanged throughout the GHB-induced SWD. Scatchard analysis revealed that all significant changes in both the GABA and BDZ sites during GHB-induced SWD were due to changes in receptor.

These data do not support the hypothesis that the GABA site is primarily involved in the genesis of SWD but raise the possibility that the BDZ site might be.

551.7 BIOLOGICAL CONSEQUENCES OF TRANSCRANIAL MAGNETIC STIMULATION IN THE MOUSE. SM. Hirsch, RG. Green, JD. Weinstein*, RJ. Freid, KR. Davie, CM. Epstein* and RA. Bakay, Department of Neurology and Neurosurgery, Emory University School of Medicine, and Department of Electrical Engineering, Georgia Institute of Technology, Atlanta, Georgia.

Transcranial magnetic stimulation (TMS) is a recently developed technique for non-invasive stimulation of cerebral cortex which has broad clinical and research applications in humans. However, there is little experimental data on physiologic and pathologic effects in animal models. Circular coils are usually used in human TMS and produce a field that is not useful for small animal studies. Our high speed magnetic stimulus utilizes a specially designed U-shaped alloy core electromagnet with a gap of 6 cm and pole faces measuring 2.5 cm. The induced field reliably stimulates human motor and somatosensory cortex and is painless as long as the poles do not directly contact the scalp. Male inbred C57BL6 mice, aged 2-4 months were individually placed on the cortical stimulator so that the head was within the gap of the U-shaped electromagnet, with the rostral-caudal axis perpendicular to the plane of the electromagnet. Peak electric field at the head position was 3.6 V/cm. Each mouse was placed in the experimental group received a daily total of approximately 1000 stimulations, in three 25-second trains of 15 Hz. Stimulation trains were delivered 1-2 minutes apart over 10-15 minutes. Animals were stimulated daily, 5 days per week for 8 weeks and a total of approximately 40,000 stimulations per animal. Sham-stimulated control mice were placed in an adjacent restraint outside the curve of the electromagnet exposing them to equivalent heat, light, noise and mechanical field. We found no effects of the sham with the gap of the electromagnet. Behavioral responses were the same in both groups of animals and consisted of a brief startle response and urination. No induced motor activity, weakness, or seizures were identified. Immediately following the final stimulation sequence, experimental and control mice were sacrificed, perfused and processed for histology. Blinded evaluations of NeuN and Fos-Heimer stains did not reveal any differences between experimental and control mice. Further comparisons utilizing electron microscopy and post-stimulation kindling rates are underway.


We have previously shown that the central medial intralaminar nucleus of the thalamus (CeM) regulates both arousal and the threshold of seizures induced by chemical convulsants. This nucleus receives direct projections from cholinergic neurons of the pontomesencephalic tegmentum. The present study investigates the effects of injections of GABA agonists into this tegmental region on seizures induced by timed continuous intraventricular pentylethenetrazol infusion.

Injections of the direct GABA agonist piperidine-4-sulfonic acid (PSA, 5 to 50 nmol in 50 nl) in the laterodorsal tegmental nucleus (LDTg, cell group Cb) and the adjacent, but more rostral pons, was within the gap of the U-shaped electromagnet, with the rostral-caudal axis perpendicular to the plane of the electromagnet. Peak electric field at the head position was 3.6 V/cm. Each mouse in the experimental group received a daily total of approximately 1000 stimulations, in three 25-second trains of 15 Hz. Stimulation trains were delivered 1-2 minutes apart over 10-15 minutes. Animals were stimulated daily, 5 days per week for 8 weeks and a total of approximately 40,000 stimulations per animal. Sham-stimulated control mice were placed in an adjacent restraint outside the curve of the electromagnet exposing them to equivalent heat, light, noise and mechanical field. We found no effects of the sham with the gap of the electromagnet. Behavioral responses were the same in both groups of animals and consisted of a brief startle response and urination. No induced motor activity, weakness, or seizures were identified. Immediately following the final stimulation sequence, experimental and control mice were sacrificed, perfused and processed for histology. Blinded evaluations of NeuN and Fos-Heimer stains did not reveal any differences between experimental and control mice. Further comparisons utilizing electron microscopy and post-stimulation kindling rates are underway.
EPILEPSY: ANIMAL MODELS

551.10
PERINATAL HYPOXIA HAS LONGTERM EFFECTS ON EEG ACTIVITY AND SEIZURE SUSCEPTIBILITY IN RATS. E.L. Jensen, C.D. Applegate, AND M. Chandy. Children's Hospital, University of 2011 E.E.G. AND Neuro-sciences Unit, N.Y. Medical Center, Rochester, N.Y. 14614.

We have shown that hypoxia-induced seizures in neonatal rats were accompanied by a 20-30 Hz spike-waves that were associated with a decrease in EEG activity. We have also observed that hypoxia-induced seizures were associated with a significant increase in the number of spikes and waves in the EEG, as well as a decrease in the frequency of the EEG activity. These findings suggest that hypoxia-induced seizures in neonatal rats are associated with a decrease in EEG activity and an increase in the number of spikes and waves in the EEG. These results are consistent with previous studies that have shown that hypoxia-induced seizures are associated with a decrease in EEG activity and an increase in the number of spikes and waves in the EEG.

551.11
IS THE ANTICONVULSANT EFFECT OF NIGRAL INFUSION OF GAMMA-VINYL-GABA (GVG) MODIFIED BY A-HISTAMINE H1 RECEPTOR IN RAT PUPS? S.G. Xu, E.F. Sperber, S.L. Mohl. Dept. of Neurology & Neuroscience, Albert Einstein College of Medicine, Bronx, NY, 10461.

Nigral infusions of GABAergic compounds have provided pharmacological evidence indicating a crucial role for the GABAergic system in the control of seizures. We have previously shown that nigral infusions of GVG suppressed flurothyl-induced seizures in rats, while perinatal administration of muscimol (A/B) receptor agonist or bicuculline (A/B) receptor antagonist was ineffective in reducing seizures. In this report, we generated a dose-response curve of the GVG effects and investigated the possible role of nigral A/B receptors in the mediation of the anticonvulsant effect of GVG. Nigral infusions of GVG at a dose of 2 μg significantly delayed the latency of flurothyl seizures compared to controls while doses >10 μg induced sedation. Bilateral nigral infusions of muscimol (100 ng) or bicuculline (100ng) reduced the anticonvulsant effect of GVG.

These findings suggest that rat pups, the optimal dose of intragastrically infused GVG against flurothyl seizures is between 5 and 10 μg/ml. There are at least two explanations for the results obtained by coadministration of the drugs. Firstly, both A/B receptor agonists and A/B receptor antagonists have the same effect, once the explanation is that the reversal of the GVG effect is due to the reduction in GABAergic receptors. Alternatively, it might be due to specific and not mediated by the GABAergic system. These results suggest that anticonvulsant effect of GVG may, in part, involve the nigral GABA A receptors.

551.14

Cortical slow potential (SPC) usually occurs during convulsions and spreading depression(SD). However, the only information we have about the occurrence of SPC in subcortical regions is that it might be associated with a decrease in EEG activity. We have shown that SPC occurs in subcortical areas and can freely influence cell activity at other regions of the brain when we stimulated a particular electrode to record SPC at different brain structures in freely moving animals. The electrode consists of a 1 cm long isolated silver-germanium electrode with 0.2 mm diameter tip, which is peeled off and immersed in 60 % CO. The Ag/AgCl tip is then coated with a silicone elastomer (elongation rate of 800%). The SPCs were recorded in gerbil by electrocorticography (PTK) and SD in rats by KCl microinjection. We recorded SPC at cortex, thalamus, striatum and hippocampus and observed SPC in all these regions using PTK and KCl injection. The SPC, which is sometimes accompanied by a decrease in EEG activity, reflects a percentage of cells which become refractory to stimulation and will possibly interfere in the firing activity of other cells. Considering the complex cell network we have in the brain we expect that this will influence the occurrence of SPC in different regions of the brain.

Financial support: CNPq and FINEP.
SUPPRESSIVE EFFECT OF INTERICTAL SPIKES ON EXPERIMENTAL SEIZURES. Z. Elazar, M. Schwartz* and G.F. Friedman*. Department of Physiology and Pharmacology, Tel-Aviv University, Tel-Aviv, 69978, Israel

An inverse correlation between the frequency of occurrence of the interictal spikes (IS) and of seizures was indicated by previous studies. Other studies also showed a similarity between the two epileptic phenomena.

We studied this problem in experiments on rats anesthetized with urethane. Penicillin epileptic foci were produced on the cortical somatosensory area. Electrical stimuli (100-500 µA, 1 ms) were delivered to the VPI nucleus. The stimulator strength was adjusted until triggering of the IS was effective. Driving of IS was displayed as a graph in which the rate of stimulation/frequency (T) was plotted as a function of the rate of stimulation. The rate of stimulation was increased until T=1, where no spontaneous IS were recorded and all IS were effective. A Suppression Rate (SR) was calculated, which seizures were suppressed for the period of stimulation was established for each experiment. The SR was close or above the T=1. The difference between the SR and the stimulation rate which gave a T=1 varied with the excitability of the cortex (depth of anesthesia) and the strength of the thalamic stimuli.

These results suggest that in certain conditions the interictal spike has an inhibitory effect on the mechanism generating the seizure. This inhibitory effect accumulates during trains of IS.

TRIUMA: SPINAL CORD, NMDA AND OTHER

DOSE RESPONSE STUDY OF NIMODIPINE IN ACUTE EXPERIMENTAL SPINAL CORD INJURY. I.B. Ross, C.H. Tator, E. Theriault. Playfair Neuroscience Unit, University of Toronto, 399 Bathurst St., Toronto, CANADA M5T 2S8.

Recent studies of nimodipine treatment in acute experimental spinal cord injury (SCI) in the rat have demonstrated its effectiveness for partially reversing post-traumatic ischemia up to 3 hours after injury. There is also evidence that it improves SCI. Potentials were recorded and measured by motor and somatosensory evoked potentials. These effects have only been seen when nimodipine is combined with either a vasopressor or a volume expander.

A study being completed in our laboratory at the present time is designed to determine if nimodipine alone, without vasopressor or volume expander, is of any benefit in the treatment of acute experimental SCI in the rat. Thirty minute infusions of 0.05, 0.25, 0.01, 0.005 or 0 mg/kg of nimodipine after injury are being assessed. Spinal cord blood flow (H2 clearance) and motor and somatosensory evoked potentials are recorded before and after injury and after drug infusion. Preliminary analysis indicates that nimodipine alone is not able to reverse post-traumatic ischemia or improve function as measured electrophysiologically.

Supported by Miles Inc. and Richard Hanson in Motion Legacy Fund.
552.5 CHANGES IN BONE FORMATION IN NEONATALLY SPINAL CORD TRANSECTED RATS. V.R. Holes, E. Gunther*, E.L. Hill, C. Cone* and E. More-Holst. The Miami Project, University of Miami, Miami, FL 33136 and NASA-Ames Research Center, Moffett Field, CA 94035.

The mechanism of bone loss following spinal cord trauma is not understood. The purpose of this study was to investigate the rate of bone formation following the transection of the spinal cord in rats that recover some function of their hindlimbs post-injury. A complete spinal cord transection was done at the T8 spinal cord level in 5 day old rats. Sham animals served as controls. Rats received either cycloheximide or demeclocycline injections (n=10) at 37, 72 and 89 days post-injury (DPL) to label bone forming during different periods and were sacrificed 90DPL. Transection of the spinal cord at the level of the tubular junction were used for bone histomorphometry. Tibial length, medullary area, and cortical area (S1DPL, and S90DPL) were significantly lower in lesioned animals compared to controls. Other bones, above and below the lesion, were evaluated for mechanical properties and trabecular bone histomorphometry. During the period t=37-90DPL, petiole bone formation rates were the same in lesioned and control animals (0.251 mm2/day), but 72-90DPL, the formation rate in lesioned animals (0.293 mm2/day) was less than that of control animals (0.319 mm2/day). This indicates that 1 month after lesion, bone is formed in a normal rate in the tibia. The difference in length and area of lesioned and control bones 2 months later may be due to an initial lag in growth immediately after lesion that is not compensated for although formation rates return to normal; a decrease in bone formation in older animals, possibly due to secondary changes (neovascularity); or a combination of these. Differences in the cross-sectional shape of the tibia in lesioned animals indicate that they may use their hindlimbs differently from normal.


A sensitive fluorometric method was modified for the evaluation of drug action upon rat spinal cord injury. Fluorescein isothiocyanate-labeled dextran (FITC-D, MW 70,000) was used as a tracer, was injected IV 2 hr before sacrifice. pH 8.2 and 0°C was used for fluorescence with FITC-D. The recovery in spinal cord was 100.1 ± 4.0% (X ± SD). The extent of FITC-D extravasation was expressed as the vascular injury index (VII).

VII = nr of FITC/mg tissue protein.

552.7 REGENERATION OF PERIPHERAL NERVES IN PRIMATES THROUGH SEMI-PERMEABLE SYNTHETIC GUIDANCE CHANNELS. M. Goddard*, R. Valentin, J. Cohen and P. Aebischer.

Section of Artificial Organs, Biomaterials, and Cellular Technology, Brown University, Providence, RI, 02912.

As part of an effort to develop devices that may improve functional recovery following surgical repair of peripheral nerve injuries, semi-permeable nerve guidance channels (AC made of the copolymer, poly(acrylonitrile vinyl chloride) were used to repair transections of the recurrent branch of the mandibular nerve in 5 cynomolgous monkeys. Nerve transections in one hand were repaired with AC channels and the recovery compared to that of identical lesions on the contralateral hand that were repaired with similarly sized silicone elastomer made of silicone elastomer (SE). Functional recovery was evaluated daily using a timed behavioral paradigm and at the conclusion of the 1 year implantation period, opening up electrophysiology. Morphologic evaluation of the regenerated nerve cables was based on myelinated axon counts. Results of the behavioral testing indicated that excellent functional recovery was achieved in this model with both guidance channel designs, however, return to pre lesion performance levels was faster with the AC devices. The electrophysiology studies demonstrated a mean combined muscle action potential and stimulus threshold of 2.9±2.3 V and 208±53.6 μA respectively for the AC channels versus 2.1±1.1 V and 252±173.6 μA respectively for the SE channels. Mean regenerated myelinated axon counts were 247±53.6 for the AC devices and 188±57.7 for the SE devices. The results of this study confirm previous rodent studies which indicated that semi-permeable AC nerve guidance channels show improved regrowth relative to impermeable channels and may hold promise for clinical repair of nerve injuries.


The neurotoxicity of glutamate was investigated quantitatively in mixed neuronal and glial spinal cord cell cultures from E12 - 13 fetal mice. Five minute exposure to 10 - 1000 μM glutamate produced widespread acute neuronal swelling, followed by neuronal degeneration over the next 24 hr (EC50 for about 100-200 μM); glia were not injured. Glutamate was neurotoxic in cultures as young as DIV 4, although greater death was produced in older cultures. A massive release of nutrients of neurons were destroyed by a 5 min exposure to 500 μM glutamate.

Acute neuronal swelling following glutamate exposure was prevented by the inclusion of extracellular calcium (1.0 mM) with or without calcium reduction in late cell death. Removal of extracellular calcium enhanced acute neuronal swelling but attenuated late neuronal death. Both acute neuronal swelling and late degeneration were selectively blocked by the noncompetitive NMDA receptor antagonist dizocilpine and by the novel competitive antagonist CGP 37849. In contrast, 100 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in the presence of 1 mM glycine produced only weak neuroprotection. 7-chloro-kynurenate also inhibited glutamate neurotoxicity, with near complete protection noted at 10 μM; protection was completely reversed by the addition of 1 mM glycine to the bathing medium. These observations suggest that glutamate is a potent and rapidly acting neurotoxin on cultured spinal cord neurons, and support involvement of excitotoxicity in acute spinal cord injury. Similar to other excitotoxic neurons, degeneration of spinal neurons induced by brief glutamate exposure is dependent on extracellular Ca2+ and the activation of NMDA receptors.


We have previously reported that temperature reduction to 17°C for 2 h significantly increased the percentage of neurons that survived amputation of a primary dendrite (15, 1.7) and increased temperature to 17°C. However, both lesioned and sham injected neurons showed dendroscopic swelling and, upon rewarming to 37°C, most swollen neuronal dendrites were retained in the cell. We conclude that dendritic swelling during HT involves the NMDA complex, and 2) whether NMDA blockade + HT increases lesioned neuron survival more than HT alone.

Mouse spinal cord neurons were grown in monolayer cultures. In Study II, a laser microbeam was used to ablate a primary dendrite from each of 10 neurons in each culture were selected for observation. In 3 groups (0, 30 min; 60 min survival in the presence of ketamine (100 μM), MK-801 (10 μM) or D-AP5 (20 μM) was added to the medium. No antagonists was added to controls. Cultures were kept at 25°C and 22°C at 37°C. NMDA antagonists reduced swelling during HT. At 37°C, 10% of the surviving neurons in each experimental group had died compared to 74% of the controls. In Study II, a laser microbeam was used to ablate a primary dendrite from each of 10 neurons in each culture (distance from soma 100 μm). After culture and the culture media was replaced with medium at 10°C, 17°C, or 37°C. D-AP5 (30 μM) was added to the medium. No antagonists was added to controls. Cultures were kept at 25°C and 22°C, at 37°C. NMDA antagonist reduced swelling during HT. At 37°C, 10% of the surviving neurons in each experimental group had died compared to 74% of the controls. In Study II, a laser microbeam was used to ablate a primary dendrite from each of 10 neurons in each culture (distance from soma 100 μm). After culture and the culture media was replaced with medium at 10°C, 17°C, or 37°C. D-AP5 (30 μM) was added to the medium. No antagonists was added to controls. Cultures were kept at 25°C and 22°C, at 37°C. NMDA antagonist reduced swelling during HT. At 37°C, 10% of the surviving neurons in each experimental group had died compared to 74% of the controls.

Supported by grants from NINDS (PO5 NS08066) and the Hillcrest Foundation of Dallas, TX, founded by Mrs. W.W. Caruth, Sr.
552.11 PRETREATMENT WITH NMDA ANTAGONISTS LIMITS RELEASE OF EXCITATORY AMINO ACIDS FOLLOWING TRAUMATIC BRAIN INJURY. S. S. Penter and A. I. Faden. Department of Neurology, V.A.M.C., San Francisco, Calif.

After central nervous system trauma, there are marked elevations in the extracellular levels of excitatory amino acids, which are believed to contribute to delayed tissue and biochemical events. Administration of NMDA antagonists reduces severity after brain or spinal cord trauma, presumably by blocking the postsynaptic NMDA receptor. In the present studies, levels of extracellular excitatory amino acids were monitored by microdialysis during, and after, a moderately severe (2.8 atmosphere) lateral fluid percussion brain injury to rats, as previously described (Science, 244:796-800, 1989). At the end of the first sampling interval (10 min) following injury (n = 6), extracellular glutamate and aspartate increased 20- and 15-fold, respectively, over controls (n = 8).

Pretreatment (15 min. prior to injury) with the non-competitive NMDA antagonist dizocilpine (1 mg/kg i.p.) or the competitive NMDA antagonist CGS 19755 (30 mg/kg i.v., n = 5) significantly attenuated the posttraumatic increase in extracellular glutamate (from 10.9 ± 2.6 (SEM) µM in dialysate from untreated animals to 51.7 ± 7.9 (SEM) µM in animals treated with CGS 19755). These results are consistent with recent in vitro experiments and suggest that dizocilpine and CGS 19755 may limit the release of glutamate and aspartate after trauma through mechanisms involving presynaptic modulation.

552.13 Intravenous Administration of Heat Shock Protein-70 (HSP-70) STEVENS PA,*, GOWER DJ Section of Neurosurgery and Anatomy, Univ. Of Oklahoma, OKC 73104.

Endogenously expressed HSP's are protective for cellular stress. Berberian et al. 1989 found that exogenous HSP-70 protected cultured cells. We have examined the fate of HSP-70 following intravenous administration in a mammal. HSP-70 was purified from bovine brain (Schmidt and Reinke, 1985). The purified protein was labeled with I-125 and the purity determined by SDS-PAGE. Anesthetized adult rats were administered 10 µg of label. HSP-70 combined with 10 UCl of TC-99M labeled albumin. Blood and tissue samples were harvested and examined. Initial counts were done in the energy range for the TC-99M. The tissue was allowed to decay for at least 12 half-lives and recounted for I-125.

The albumin space for each organ was determined and the amount of HSP-70 above the background accounted for by vascular space was calculated. Liver 171.04ng/g, spleen 183 ng/g, kidney 63.33ng/g, and lung 26.72ng/g were the only organs to uptake HSP-70. The brain and spinal cord had no selective uptake above vascular space. These data suggest that HSP-70 may be administered intravenously but the usefulness in CNS disease will be limited by an intact blood brain barrier.


We are developing a technique for measuring rheological properties of individual neuronal cells. We plan to use this technique to determine how neurons might be affected by mechanical forces, such as those experienced in physical trauma. The technique will also enable us to quantitate the adhesive properties of neuronal cell adhesion molecules. Dissociated neuronal cells are grown on substrates of varying adhesive properties, or on monolayers of glial cells. The cells are transferred to, or grown in, a chamber which is attached to a pump capable of delivering a controlled flow of solutions of chosen viscosities. This chamber is placed on a microscope set up for videotape recordings. The shear stress necessary to deform, detach or disrupt individual cells is determined. Cells are seeded onto patches of purified neuronal cell adhesion molecules. The relative adhesive strength of the molecule is determined by the shear force required to dislodge the cell. This technique can be utilized to quantify the adhesive regions of proteolytic fragments or deletion and point mutation products of cell adhesion molecules.


In this study, the acute evolution of the neurotoxic effects of MPTP were evaluated in the monkey model. Squirrel monkeys (n=17) were given a single injection of MPTP (2.5 mg/kg). The behavioral changes were monitored and the concentrations of dopamine (DA), dihydropyridolcarboxylic acid and homovanillic acid were measured in striatal and nigra tissue 1, 3, 5 and 10 days after drug administration. Neuropathological examination of two animals 8 and 9 days after MPTP revealed severe core cell destruction in the caudate. MPTP produced severe parkinsonian behavior changes in all animals after 1 day. Interestingly, although 50-75% reductions were observed in the substantia nigra and pars compacta, the changes were not reduced in the caudate and, actually, was increased in the putamen at these time points. Five and 10 days after MPTP, nigral DA depletion remained greater than 70%, but substantia nigra was reduced 50-85%. At these time points, the putamen was always more affected than the caudate. DA metabolites were decreased in both the substantia nigra and striatum at all time points. These results indicate that: (a) nigral cell bodies may represent an important initial site of MPTP-induced damage; and (b) the interregional pattern of striatal DA deficits caused by MPTP is similar to that seen in idiopathic Parkinson's disease.


This study quantifies the tyrosine hydroxylase - immunoreactive (TH-IR) cells in the A8-A9 areas in normal and MPTP treated monkeys. Monkeys (n=7) were individually caged and given free access to food and water. Procedures were approved by NINDS Animal Care and Use committee.

Four monkeys received MPTP (0.4 mg/kg by intracarotid injection) and developed hemiparkinsonism. Four to six months after treatment these three and normal monkeys were euthanized. Brain tissue was fixed at 25 um and every 10th section stained for TH-IR. Adjacent sections were stained with thionin and for dopamine beta hydroxylase-IR. TH-IR cells were decreased in MPTP-treated monkeys by 88-96% in A9 and 60-72% in A10. The medial portion of A10 was relatively spared. These cells are likely to represent the population from which sprouting dopaminergic fibers; seen in the caudate of transplanted monkeys, arise. Preliminary data indicates areas A8 & A9-A13 show no significant depletion after MPTP treatment.

The neuropathological changes indicative of parkinsonian disease are well known. The use of a small animal model of this disorder requires adequate assessment of the quality, severity, duration of the effects, and complications of MPTP treatment.

Sixty Cercopithecus aethiops sabaeus were treated with MPTP (0.3-10 mg/kg) 1-5 times, cum. dose 1.2-2.4. Trained observers 'scored' and 'rated' behavior twice daily (5-10 min, 3-6 times per day) for 7-14 weeks after MPTP-treatment (e.g., freez in immobility, chew, yaw, or scored per 5 sec duration (e.g., eating, drinking). Behavior were also quantified on a rating scale of 0-5 (e.g., severity of movement, limb support, tremor) and after 'challenging' (e.g., threat). Individual behaviors were analyzed, as well as summary scores for parkinsonian, healthy, anxiety, and arousal-related behavior. Levels of CSF HVA were determined in some samples. Performance was also examined on 'executive' or cognitive tasks.

MPTP resulted in behavior not observed in control subjects (i.e., tremor, freezing, immobility, eating problems, and lack of movement) and reduced signs of healthy, arousal, and anxiety associated behavior. The parkinsonian summary score was used to categorize the severity of MPTP deficits. Idiosyncratic doses and treatment schedules led to behavioral deficits ranging from extremely mild to severe. All MPTP subjects had reduced levels of HVA in CSF at sacrifice. Medical and physiologic complications analogous to those observed in parkinsonian patients were also observed.

524022, Anim Res Found, St Kitts Biomed Res Found, DER - RSA MI84064S


Female squirrel monkeys (7 pairs, 550-750 g) received daily i.p. injections of n-propyl-4-phenyl-2,3,6-trihydropyridine (MPTP; 0.63-1.25 mg/kg) for 9-14 days, beginning on the day of either a bilateral 6-hydroxydopamine lesion of the locus coeruleus (LC) or a sham operation. Seven to 8 weeks after the start of MPTP treatment, and in marked contrast to the sham-operated animals, the LC-lesioned monkeys showed a profound parkinsonian deficit (HVA levels >84% depletion), extensive cell loss in the substantia nigra, and little or no recovery from the parkinsonian motor symptoms (tremor, bradykinesia, hypokinesia and reduced blink rate) induced by MPTP.

These neurologic, biochemical and histologic assessments indicate that lesioning of the LC impairs the recovery which typically occurs from the parkinsonian manifestations induced by MPTP in squirrel monkeys. Results of this study support the hypothesis (F.C. Colpaert, Neuropharmacol. 26: 1431-1987) that deficient LC-noradrenergic mechanisms might play an underlying role in the progression of Parkinson's disease.

553.7 PHARMACOLOGICAL MANIPULATION OF COGNITIVE PERFORMANCE AND IMPERSONIENCE IN MOTOR ASYMPTOMATIC MONKEYS CHRONICALLY EXPOSED TO MPTP. C.J. Kovelman*, J.L. and J.S. Schneider, Dept. of Neurology, Hahonan University School of Medicine, Philadelphia, PA. 19120

Monkeys exposed to low doses of MPTP over several months have been shown previously to develop specific cognitive deficits in the absence of overt motor disturbances. In the present study, three M. nemestrina monkeys were trained to perform a two-choice (2DO) delayed matching-to-sample (DMS) task. In all animals, performance was the best at tonic levels of stimulation, and sensitive to inhibition. In time, performance of DMS, OR, and DR was also disrupted. In contrast, VD performance remained intact. In all animals, performance improving the previously learned task incorrectly, so animals demonstrated striking performance during attempted task performance with many incomplete trials recorded. On the DMS task, after numerous injections, animals consistently failed to retrieve food that was not readily accessible via direct line of sight. These animals continue to do poorly and fail to make appropriate motor responses to the stimuli. The findings are consistent with the hypothesis (F.C. Colpaert, Neuropharmacol. 26: 1431-1987) that deficient LC-noradrenergic mechanisms might play an underlying role in the progression of Parkinson's disease.
553.11

The role of vitamin E (VIT E) deficiency on striatal dopaminergic (DA) system function was studied in rats. 6-OHDA unilateral lesions of the substantia nigra (SN) were induced in 20 Sprague-Dawley rats (250-300 g) which were then tested 14 days later for turning behavior in response to i.p. injections of the DA agonist apomorphine (AP). To probe the role of vitamin E deficiency on the dopaminergic system, we hypothesized that vitamin E deficiency would result in an even more rapid loss due to oxidative damage and that this would result in a more rapid decrease in turning rate. Results: There were no differences in the turning rates, although VIT E deficient rats exhibited a trend towards increased turning in response to AMP (p = 0.12). This result is consistent with our other findings of significantly increased striatal DA levels in rats on a VIT E deficient diet.

553.12

Since the N[3]-methyl-beta-carboline (3Me-BC), 3Me-harman, approaches MPP+ in potency of mitochondrial respiration inhibition in vitro (Hoppel et al., 1987), we examined a number of physiologically possible 3Me-BCs and dihydro-BCs (2Me-BCs) for effects on o2 utilization by rat liver mitochondria. Following 6 min pre-incubations, 3Me-harman, 3Me-harmine and 3Me-harmaline had IC50's comparable to MPP+, m-norharman and 2Me-harmine were only weak inhibitors. Like MPP+, inhibition by 2Me-DHBC/BCs was augmented by tetraphenylboron and reversed by DNP, consistent with the involvement of cationic 2Me-DHBC/BC forms. However, the inhibitory time courses and DNP uncoupling patterns were dissimilar to MPP+, indicating a role for uncoupled 2Me-DHBC/BC forms as well. The 3Me-BCs tautomers may arise from deprotonation of the indole N, since 2,9-dimethyl-norharman, a cationic BC that cannot deprotonate, had an inhibitory time course and response to DNP that resembled MPP+ rather than other BCs. Unlike MPP+, the 2Me-DHBC/BCs blocked mucroate-supported respiration almost as effectively as non-DNP-uncoupled respiration. The findings are consistent with the hypothesized role of endogenous 2Me-DHBC/BCs in the etiology of Parkinson's disease. (Support: NIH NS23891).

553.13

In ongoing studies of the neurotoxic capabilities of MPP+-related beta-carboline (BC) analogs, it was found that the PC12 cell cytocidality exerted by cationic 2-[N]-methyl BCs is significantly enhanced by further methylation of the indole-9 nitrogen. This was supported by initial results with isolated mitochondria in which 2Me-norharman, a very weak inhibitor of respiration, became more effective than MPP+ when the 9-6 proton was replaced by methyl. Previously in PC12 cells, we observed that 2Me-norharman produced negligible release of LDH or decrements in cell protein and dopamine uptake (indicators of cell toxicity) in 4 day pre-incubations. 2,9-dime-norharman was equipotent with MPP+ in all three measurements after 2 days. Similarly, 2,9-dime-harmane was identical with MPP+ and 2,9-dime-norharman, whereas 2Me-harmane showed minimal toxicity at 2 days. A 3rd compound, 2,9-dime-harmane, achieved toxicity equal to MPP+ at 4 rather than 2 days, while the unmethylated 2Me-harmane was without effect throughout. It metabolically feasible, replacement of the indole proton with a methyl or other group may be an effective neuroprotective strategy for endogenously-derived 2Me-BCs. (Support: NIH NS23891).

553.10
CHANGES IN DOPAMINE (DA) RELEASE IN VITRO FROM THE CORPUS STRIATUM (CS) OF YOUNG AND AGED RATS AS A FUNCTION OF INFLUENCE MODE OF L-DOPA, K+ AND AMPHETAMINE (AMPH). J. McDermott, D. Diurcen & V. Bantjes. Dept. of Biophysics, University of Medicine-Garle Hospital and Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

We have reported differences in DA release in vitro from the CS of young versus aged rats in response to a pulse infusion of L-DOPA (Diurcen, et al. Exp. Neurol. 106:259, 1989). In the present study, we examined the effects of these fragments from young (Yr=4-6 months) and aged (Aa=18-24 months) male rats were superfused in vitro and received either two 10 min saline or (10 min sodium acetate) continuous (120 min) infusions of L-DOPA (50ug, K+ (30 mm) or AMPH (10ug). For both young and aged rats the peak response ratios of the second first response for the two test peptides were virtually identical for K+ (Yr=3.3, Aa=4.4, 0.057 and AMPH (Yr=0.46±0.08, Aa=0.46±0.03, N=6). However, there was a significant difference (p<0.05) in response to L-DOPA pulse infusions (Yr=2.8±0.3, Aa=2.4±0.17, N=7). Overall DA release to continuous infusions (Yr=12, Aa=12) were greater in young vs aged CS for K+ (Y=4.6, 0.018, A=21.2±3.5, N=6) and AMPH (Y=9.7±3.13, A=52.58±5, N=6). In contrast, a continuous infusion of L-DOPA resulted in a greater release of DA from aged CS (258±24.6±3, N=7 vs A=4±0.04±0.5, N=6). These results demonstrate influence mode dependent differences in DA release from the CS of young versus aged rats in response to these secretagogues.

553.9
DEGENERATIVE DISEASE-PARKINSON’S: MPTP MONKEYS AND RODENTS. FRIDAY AM

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
553.17 EXCESSIVE METHYLATION AND PARKINSON'S DISEASE: L-DOPA EFFECTS. B. Benson, J. Clark, C. Chartoff. Dept. of Physiology, Medical University of Louisville, Louisville, KY 40292.

When injected into the brain of rats and mice, S-adenosylmethionine (SAM) causes Parkinson's disease (PD)-like symptoms, such as tremors, dyskinesia, and abnormal movements. The major treatment for PD is L-dopa. L-dopa is metabolized to dopamine (DA) and reacts with SAM. Therefore, the increase in DA as well as the depletion of SAM may elicit the positive benefits of L-dopa therapy. However, the lack of efficacy from prolonged L-dopa therapy may be due to a rebound increase in SAM via activation of methionine adenosyltransferase (MAT), the enzyme that synthesizes SAM from methionine and ATP. We tested this hypothesis by administering (p.o.) saline, or L-dopa to rats and mice and using assay for MAO-A.

About 73% and 53% of the activity of MAO was observed in the insoluble fraction of the brain and liver, respectively. L-dopa increased the activity of MAO in the brain by 52% and 37% in the 100 and 500 mg/kg, as compared to saline, treated rats. L-dopa, 50 mg/kg, increased the MAO activity by 37% in mouse brain. No significant changes occurred in the liver of rats or mice. Since l-dopa increases MAO-A, the inability of changes in PD therapy may be explained partly by an increased MAO activity, which will increase SAM and create a vicious cycle. L-dopa will react with both L-dopa and DA to produce methylated products. It has been shown that high plasma levels of methyl-dopa were observed in patients exhibiting l-dopa induced dyskinesia; also, in animals, methyl-DA and SAM caused motor deficits antagonistic to l-dopa.

(Supported by NIH RR0032, NSF RH704121 and NSF 8714805.)

553.18 A MONOClonAL ANTIBODY (MAb) TO DARP (DOPAMINE RELEASING PROTEIN) SELECTIVELY KILLS NEURONS IN CULTURE FROM THE MESENCEPHALON AND REDUCES Dopamine (DA) LEVELS IN THE STRIATUM (CS) AND HYPOTHALAMS OF IMMATURE RATS. V. D. Ramirez, D. G. Dielen, S. Kothanathan, S. Miklasz*, and E. Marcus*, Department of Cell Biology and Hormone, Ltd, and Chiron Corp.

Cell cultures from mesencephalon (M) and diencephalon (D) of 17-18 day-old rat embryos were prepared. On day 9, substances dissolved in minimal essential medium were added. On day 16, cells were counted. A dramatic arrest of growth and neuronal differentiation of M and D cultures that received the MAb (14 pg/well) was observed. The number of neurons/1.7mm2 were: 14.6±5.4, n=24 controls vs 2.05±0.5, n=24 MAb-treated. In the D cultures were: 8.1±1.7 n=9 control vs 6.8±1.3, n=12 MAb-treated. Recent born pups were injected subcutaneously daily for 10d with MAB-E, 20 or 5 μg/rat or with 20 μg y-globulin. On day 11 of 25 DA levels were measured in the CS or hypothalamus. The MAB decreased DA concentrations in the CS on day 11 (3.9±0.26, 3.7±0.19 and 2.6±0.16 mg/mg, n=5-6 for controls, 5 μg and 20 μg groups, respectively) and in the hypothalamus on day 25 (84±570 controls vs 48±141 pg/mg 20 μg/MAb-treated, n=5). These data suggest that an endogenous neurotrophic factor is released from cells of M cultures that is essential for their growth and neuronal differentiation. The anti-DARP MAb blocks this action and is also capable of reducing DA levels from the CS and the hypothalamus.
554.3 IMMUNOLOGICAL REACTIONS IN AMYOTROPHIC LATERAL SCLEROSIS BRAIN AND SPINAL CORD TISSUE. T. Kawamata*, H. Akiyama, T. Yamada, P. L. McGee, E. G. McGee, Kineman Laboratory of Neuroscience, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5. Expression of proteins associated with immune function was examined immunohistochemically in postmortem brain and spinal cord tissue from patients with amyotrophic lateral sclerosis (ALS). These included the leukocyte surface antigens CD3, CD4, CD8, and FcγRII; complement receptors CR3 and CR4; complement proteins C3d and C4d; LFA-1 and its ligand ICAM-1; and HLA-DR. Reactive microglia expressed high levels of LFA-1, CD4d and HLA-DR. They were found in abundance in the primary motor cortex, motor nuclei of the brain stem and the anterior horn of the spinal cord. Throughgoing reactive microglia and macrophage tracts from subcortical white matter of the motor cortex to the anterior and the lateral funnel of the spinal cord, the cells were lipid filled having the morphology of classical fat granule cells. A significant number of lymphocytes reactive for LCA, CD3 and CD8 were observed marginating along the walls of blood vessels and invading the parenchyma of inflamed areas. Clusters of C3d and C4d coated fibres were frequently associated with oligodendroglia. We describe these as complement-activated oligaedendroglia. Labelling of the central nervous system components with complement proteins might be evidence of opsonization, which could stimulate phagocytosis by reactive microglia/macrophages bearing complement receptors. Expression of HLA-DR by these phagocytes indicates their capability of antigen presentation to T-lymphocytes, possibly followed by the production of specific antibodies.

554.4 ISOLATION OF MOTORNEURONS AND CHARACTERIZATION OF RNA AND NEURONAL INTERMEDIATE FILAMENT PROTEINS FROM AUTOPSY VENTRAL SPINAL CORDS OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS. B.A. Brody, D.D. Kelley-Geraghty* and B. Jubelt*, Northwestern Univ. Sch. of Med., Chicago, IL 60611. A variety of methods have been utilized to isolate large numbers of neurons from the brains of adult animals or from human autopsy material. A quantitative analysis of motor neuron RNA has been reported for bovine spinal cord (Cappez- Covey, P. and Nclwan, D.L., J. Neurochem., 25:517, 1975). The resulting membrane defects caused by mechanical dissociation of neurons as well as postmortem autolysis may obtaining intact neurons from human autopsy tissue particularly difficult. We are able to obtain highly enriched fractions of neurons from the ventral horn of frozen spinal cords from both patients with amyotrophic lateral sclerosis (ALS) and control patients with nonneurological disease. The neuronal yield from ALS patients is approxi- mately 1/10 that of the controls. We have been successful with autopsy material with as much as an 18 hour postmortem interval or frozen for as long as 4 years. We describe immunoperoxidase and immunofluorescence studies on these neuronal fractions utilizing antibodies to high molecular weight and to 57kDa neurofilament proteins. We have also quantitatively isolated nondegraded RNA from frozen ventral spinal cord tissue of both ALS and neurologically normal patients. These studies will provide a basis for biochemical, immunological, and biological characterization of ALS versus normal tissue.

554.5 CELLULAR CHANGES IN THE MOTOR CORTEX OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS. K. J. Kasarskis, H. Liu*, H. Wang* and S. Khare*. Dept Neuro, VA and Univ of Kentucky Health Sciences Center, Lexington, KY 40536-0084. Amyotrophic lateral sclerosis (ALS) is characterized by selective death of motor neurons in the spinal cord. Pyramidal tract involvement and pathologic changes in the motor cortex are also recognized. However the extent of neuronal loss in precentral cortex has not been quantitated in ALS. Four patients dying with ALS and 4 age-matched controls were studied. A series of images, comprising a "core" of cortex taken from the leg region of Brodmann's area 4 and measuring 270 x 3400 sq. microns, were analyzed by a computerized image analysis system. A total of 4 cores were examined from each subject. The apparent area and position of each cell relative to the pial surface was determined. The total number of cells increased by 197 in ALS cortex, reflecting primarily a 30% increase in the number of glial cells. However neurons measuring >100 sq. microns, were reduced by 50% in lamina V of ALS cortex. These data indicate that significant neuronal loss occurs in lamina V of Brodmann's area 4 in ALS. Changes of similar magnitude, if present in other cortical regions which contribute to the pyramidal tract, may explain the cortical hypometabolism seen in PET scanning of ALS patients. (Supported by NS 2356J).

554.6 Tubulin Distribution is Altered in Spinal Cord Perikarya of the Motor Neuron degeneration (Mnd) mouse. L. Callahan and J.E. Mazurkiewicz. Anatomy, Cell Biology, Neurobiology, Albany Med. Coll., Albany, NY 12208. We previously reported that neurofilaments were abnormally distributed in spinal cord perikarya of the Motor neuron degeneration (Mnd) mouse, an animal model for human motor neuron disease. The study reported here investigated the distribution of tubulin, another major cytoskeletal element in neurons. An immunoperoxidase technique using antibodies to polymer or dimeric tubulin was employed to analyse lumbar spinal cord perikarya in transverse and horizontal 30um vibratome sections. One severe and two moderate stage Mnd and two age-matched controls were examined. In control perikarya, immunoreaction product for tubulin was distributed throughout the cytoplasm. Immunoreactivity of the peripheral cytoplasm was increased relative to the inner cyto- plasm. In contrast, perikarya of Mnd contained prominent cytoplasmic regions devoid of tubulin immunoreactivity. This distribution was similar to that found for neurofilaments in affected cells in Mnd. The affected neurons were found pre- dominantly in one lamina, suggesting a selection of neurons may be occurring in this disease. The lamina containing most of the affected neurons was lamina IX, the region containing alpha motorneurons. The alteration in the distribution of tubulin and neurofilament proteins suggests that changes in cytoskeletal function, including axonal transport, may play a role in the pathogenesis of the disease. (Supported by NIH NS24426 and the ALS Association)

554.7 BRAIN UPTAKE AND DISTRIBUTION KINETICS OF BETA-N-METHYL AMINO-L-ALANINE IN THE RAT. Q.S. Smith, P. Pearson*, N. Villacreses*, L. Wyatt, L. Markley, L. Koplin, and M. Duncanson. Lab. of Neurosciences, NIA; Intramural Res. Program, NINDS; and 25A8, NIH, Bethesda, MD 20892. Beta-N-methlyalamino-l-alanine (BMAA) is a neurotoxic nonprotein amino acid present in crops. It is thought to be implicated in the pathogenesis of the amyotrophic lateral sclerosis-parkinsonism dementia complex of the western Pacific. Using autoradiography of brain tissue, BMAA uptake (25-400 mg/kg) was administered to rats either acutely or chronically, and then plasma and brain concentrations were determined at various times thereafter by GC-MS. Following single brain injections, BMAA was cleared from plasma in a rapid distribution phase (Vd = 21.5 L/kg) followed by a slower elimination phase (t1/2 = 1 day). Brain uptake rate into brain at a low rate (PS = 2.5 + 0.2 x 10^-3 ml/g/min) mediated in part by the large neutral amino acid carrier of the blood-brain barrier. Brain levels peaked within 2 hours after injection and then declined with a t1/2 similar to that of plasma. After two weeks of continuous infusion (100 mg/kg/day), steady-state brain concentration was reached. The brain concentrations were about 20-30 ug/ml greater than those of plasma. The regions suggest that BMAA may reach potentially toxic levels in brain (>300 Bq/mg) following large doses. However, acute doses are orders of magnitude greater than those available from diet or medical use of caycids.

SOCIETY FOR NEUROSCIENCE ABSTRACTS, VOLUME 16, 1990
554.10 PROTEIN KINASE C ACTIVITY IN CEREBRAL CORTEX OF CATS WITH GM, GANGLIOSIDOSIS. G. Shanker, H. J. Baker* and A. J. Perrone. Department of Comparative Medicine, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

GM, gangliosidosis is a lysosomal disease due to reduced activity of β-galactosidase resulting in decreased hydrolysis and lysosomal accumulation of glycolipids, glycoproteins, and other metabolites in brain, liver and other tissues. Pathogenesis of neuronal dysfunction in this and other lysosomal diseases is not understood. Our previous studies using feline models of the gangliosidoses indicate defective transmembrane signal transduction, including calcium dyshomeostasis. In vitro studies suggest that protein kinase C (PKC) inhibition by sphingolipids may play a role in these diseases. We measured calcium dependent PKC activity in cerebral cortex of cats with gangliosidosis, age-matched normal siblings, and unrelated normal controls using the Amerham assay procedure. Tissue preparations included whole brain homogenate, F2 fraction, synaptosomes and cytosol. PKC activity in unrelated normal cat brain was homogenous 80±5 pmol/min/mg protein (F<0.05;

554.11 3H-PRAZOSIN BINDING IS REDUCED IN BRAIN OF PATIENTS WITH NARCOLEPSY. L.C. Dixon, M. Merglak*, B. Hornykiewicz and M.D. Fisch. Clarke Institute of Psychiatry, and Sunnybrook Hospital, Toronto, Canada.

Narcolepsy is a sleep disorder characterized by sleep attacks, cataplexy, sleep paralysis and hypnagogic hallucinations. Studies in human narcolepsy and its animal (canine) model have demonstrated that treatment with prazosin, an alpha-1-adrenergic receptor antagonist, increases cataplexy and sleep time. We measured 3H-prazosin binding (0.2mM) as well as levels of noradrenaline and its metabolite MHPG in four brain areas in autopsied brain of three narcoleptic patients and five age-matched controls. Binding was significantly decreased in the frontal cortex (r=0.13, p<0.05) and amygdala (r=0.63, p<0.05) but was normal in caudate and putamen. Although noradrenaline levels were normal in all brain areas studied, in frontal cortex MHPG levels (r=0.13, p<0.05) and turnover ratio MHPG/noradrenaline (r=0.13, p<0.05) were elevated, suggesting increased activity of locus caeruleus noradrenergic neurones and consequent down regulation of the post synaptic alpha-1 receptor. We suggest that altered brain noradrenergic mechanisms may be involved in the pathophysiology of human narcolepsy. (Supported by the American Narcolepsy Association).

554.12 IN SITU HYBRIDISATION STUDIES OF SYMPATHETIC GANGLIA IN MULTIPLE SYSTEM ATROPHY AND PURE AUTONOMIC FAILURE. Q.F. Foster and S.L. Lightman. Medical Unit, Westminster Hospital, 17 Page St, London SW1P 2AP U.K.

Although sympathetic ganglion cell numbers are reduced in Pure Autonomic Failure (PAF) but seem to be maintained in Multiple System Atrophy (MSA) (Matthews 1988), we know little about the behaviour of the residual ganglion cells in either disorder. Messenger RNA (mRNA) is relatively stable post-mortem and we have been able to apply in situ hybridisation histochemistry to the study of cell function in human sympathetic ganglia in PAF and MSA. Eight micron frozen sections of sympathetic ganglia from MSA, PAF and control subjects were studied using probes directed against mRNA encoding tyrosine hydroxylase (TH), neuropeptide Y (NPY), the structural protein beta-tubulin (BT). Preliminary studies show reduced TH probe binding in the remaining ganglion cells in the PAF tissue studied, but well maintained levels in cells from MSA ganglia, with similar changes in NPY and BT probe binding.

Our initial studies suggest that ganglion cell biosynthesis of neurotransmitter and microtubular proteins is maintained in MSA but may be greatly reduced in the residual ganglion cells in PAF. Further work is underway to quantify these changes.

555.1 GAP-43 mRNA LEVELS IN ASSOCIATION CORTEX DURING NORMAL AGING AND ALZHEIMER’S DISEASE. P.D. Coleman, A.B. Wadham*, K.E. Rogers, Dept. of Neurobiology and Anatomy, University of Rochester, Rochester, NY. 14642

GAP-43 mRNA has been found to be abundant in developing neurons as well as regenerating neurons. We have suggested that GAP-43 may be used as a marker of neuronal plasticity in aging brain, and conversely, Alzheimer’s Disease (AD) may represent a loss of neuronal plasticity. Thus, mRNA levels of GAP-43 may reflect the degree of neuronal plasticity. RNA from frontal association cortex of AD patients and age-matched controls was isolated, cDNA was checked for integrity by Northern blot analysis. Four serial dilutions of each sample were bound to a nylon membrane and hybridized under saturating conditions to a probe representing the entire coding region of rat GAP-43. Results showed no difference between AD and control samples in total yield of mRNA per gram of tissue obtained from each case. In normal aging, GAP-43 message levels decreased by approximately 47% between 50 and 70 years of age and did not significantly decline thereafter. GAP-43 message levels in AD samples were not significantly different from the levels found in normal age-matched controls. (Supported by AG 09016, AG 01121, AG 03644, AG 00107, PRG-89-120)

555.2 INCREASED SPECTRIN BREAKDOWN IN FIBROBLASTS FROM AGED AND ALZHEIMER DONORS. C. Peterson*, P. Vanderklish*, P. Seubert*, C. Coman* and G Lynch. Dept. Psychobiology & Bionomics Center of Learning and Memory, University of California, Irvine, CA 92717

Several lines of evidence suggest that calcium homeostasis is altered by aging and Alzheimer’s disease. For example it has been previously shown that there are deficits in calcium uptake and cytosolic free calcium but not increases in bound calcium in fibroblasts from aged and Alzheimer donors (Ann N Y Acad Sci 568: 191, 1990). The present study suggests that spectrin breakdown may be mediated by a rise in cytosolic calcium in fibroblasts from young, aged and Alzheimer donors. Cultured human fibroblasts from young (21±1.2 yrs), aged (61±1.3 yrs) and Alzheimer (62.3±1.2 yrs) donors were obtained from the NIA Aging Cell Repository. Cells that had been deprived of serum for twenty-four hours were resuspended to 1% serum for 10 min. Electrophoretically separated proteins were transferred to nitrocellulose paper and spectrin breakdown products were immunodetected. The concentration of nitrocellulose paper of spectra breakdown products was greatest in fibroblasts from Alzheimer donors (231±71.4%) and less in aged controls (183±15.6%) as compared to young donors (102±8.3%). Changes in fibroblast activities could not account for these alterations. Thus, abnormal calcium mediated proteolysis may contribute to the altered cytoskeletal dynamics that occur during aging and Alzheimer’s disease. Supported in part by AG07865.

555.3 DISTRIBUTION OF PROTEASE NEXIN-1 (PN-1) mRNA IN RAT BRAIN AND ITS RESPONSE TO LESION. A. H. Alavi, D. Johnson*, K. H. Sherry, AND S. M. Corwin, Dept. of Psychology, University of California, Irvine, CA 92717

Protease nexin-1 is a serine protease inhibitor which shows neurite outgrowth-promoting activity in vitro. These properties suggest that PN-1 may be involved in the etiology of Alzheimer’s disease (AD) since PN-1 protein is reduced in AD brains. Thus, AD appears to be associated with an alteration of protease and protease inhibitors, possibly in part due to a breakdown of the blood brain barrier. In this study we have studied the cellular origin of PN-1 and the effect of injury on the expression of PN-1 mRNA in the hippocampus and cortical areas severely impaired by AD. PN-1 mRNA was labeled with biotin-40S base anti-sense RNA probe for in situ hybridization. The hippocampus was deafferented either by transecting the fimbria-fornix or by electrochemically lesioning the hippocampus. PN-1 mRNA hybridization was observed to be reduced in the hippocampus of animals lesioned in the dentate gyrus and pyramidal cell layer, cerebral cortex layer III, olfactory bulb periglomerular area. After either lesion, there was a transient increase (days 1 through 5) in PN-1 mRNA labeling density in the hippocampus ipsilateral to the lesion. There was not an exact topographical correlation between deafferented areas and lesion effect. This suggests that PN-1 can be induced as a general response to trauma as a possible mechanism to protect the brain and mobilize growth related events.

555.4 QUANTITATIVE IMMUNOREACTIVITY OF ALZHEIMER-LIKE ANTIGENS INCREASED BY ELDERLY HUMAN SERUM TREATMENT OF CULTURED NEURONS. G.J. Bremer, B.K. Mihalik and J.R. Ashford, Southern Illinois University, Carbondale, IL 62901

The mechanism for the development of distinct types of lesions in the Alzheimer disease (AD) brain and other changes outside the brain in patients with AD is unknown. The possibility that unregulated proteases in serum and other secretions in culture media contribute to the development of lesions was investigated. Serum from AD patients and their spouses, but not young adult human or fetal bovine, each increased three molecular markers characteristic of Alzheimer senile plaques and neurofibrillary tangles: Aβ-50, β-amyloid and MAP2. By quantitative immunofluorescence, neurological exposure to the elderly human sera produced 2.5 to 3.8 fold increases in all areas/cell and 17 to 37% increases in brightness of these three markers relative to no serum exposure. Anti-β-amyloid immunogold labeling and whole-mount electron microscopy revealed immunoactivity associated with extracellular fibrillar deposits, adjacent to neurons in samples treated with elderly human sera which was reduced and restricted to apical dendrites in samples treated with young sera. These studies direct attention toward a common mechanism of induction of both plaques and tangles in AD. Supported by the Pearson Family Foundation and SISBM.


Wandering behavior in Alzheimer’s Disease is one of the most problematic characteristics of the disease. The human literature suggests that there may be two subtypes of wandering, goal-directed and non-goal-directed. The present study attempted to dissociate the two subtypes in an animal model using the Morris Water Maze. Twenty-nine Long-Evans hooded rats were divided into two groups. The experimental group (N=9) received 1 μL of physiological saline. The animals were trained for six days (36 trials) in the water maze. The lesioned animals were significantly impaired in their ability to locate the goal in comparison to the control animals. Furthermore, the lesioned group itself exhibited marked behavior in goal-finding behavior. The moderately impaired lesioned group failed to find the platform 40% of the time in comparison to the severely impaired group that failed to locate the platform 90% of the time. It is suggested that the behavioral impairment of the moderately impaired lesioned group mimics the spatial disorientation of the goal-directed wandering observed in the Alzheimer patient. The hyperactive, motor-disinhibited behavior of the severely impaired group mimics the non-goal-directed wandering.

555.6 CYTOCHROME OXIDASE INHIBITION IMPAIRS LEARNING AND HIPPOCAMPAL PLASTICITY: IMPLICATIONS FOR ALZHEIMER’S DISEASE. M.C. Bennett, D.M. Diamond, S.L. Striker*, J.K. Parks* and W.P. Parker. Departments of Neurology and Pharmacology, UCHSC & VAMC, Denver, CO 80262

In recent work, Parker et al. (Neurobiol. Dis.) reported a selective deficiency in the respiratory enzyme cytochrome oxidase (Complex IV) in blood platelets of Alzheimer’s (AD) patients. This finding raises the possibility that a defect in the electron transport chain may play a role in the etiology of AD. Therefore, we tested the hypothesis that selective depletions of cytochrome oxidase impair learning and physiological plasticity.

Adult male rats were each implanted with an Alzet minipump (2M1) containing saline (CONTROL) or 160 mg/ml azide (AZIDE). On a shuttle avoidance task, AZIDE rats implanted 6-9 days prior to training did not show a learning curve and had longer escape latencies than did CONTROLS. This learning deficit could not be attributed to a sensory or a motor impairment. For the Morris water maze task, long-term (7 day) but not short-term (2 day) AZIDE treatment slowed the rate of acquisition. Finally, AZIDE rats were significantly impaired in their capacity to develop hippocampal LTP.

These data indicate that chronic, selective depletions of cytochrome oxidase by the concentration of the 150 and 155 kD spectrin breakdown products may contribute to the altered cytoskeletal dynamics that occur during aging and Alzheimer’s disease. Supported in part by AG07865.
555.7 ALZHEIMER’S DISEASE: MODELS

555.7.1 ALZHEIMER-INDUCED NEURITE OUTGROWTH IN CULTURED HIPPOCAMPAL NEURONS RESEMBLES NEURITE SPROUTING SEEN IN AGING AND ALZHEIMER’s DISEASE. E. Seneja and J.E. Leduc. Department of Veterinary Anatomy, Iowa State University, Ames, IA 50011.

It has been shown that the aluminum content in the human brain increases with age (up to 25 μM), and it is particularly high in those with Alzheimer’s disease (up to 910 μM). We found that 100 μM aluminum in culture induced extensive neurite outgrowth (i.e., elongation and branching of neurites) and sprouting (i.e., outgrowth of filliform-like processes from neurites) in some hippocampal neurons derived from Brazilian short-tailed opossum. Such neurite changes occurred within 24 hours of aluminum exposure. Neurites exposed to aluminum appeared to grow with no clear direction. Further, there was no statistical difference in neurite length between neurons exposed to aluminum for 24 hours versus four days. Some neurones also displayed sprouting of neurites. Such sprouting always originated from a globular enlargement at the focal area of the neurite along the neurite shaft at terminal end of the neurite. It appears that brief exposure to low level aluminum is sufficient to promote extensive neurite elongation and sprouting in some hippocampal neurones.

555.8 PERSISTENT CHOLINERGIC INNERVATION OF CEREBRAL CORTEX DESPITE DESTRUCTION OF CORTICAL NEURONS. S.L. Minet & P. Dwyer.* Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461

A controversial theory in Alzheimer’s disease research posits that degeneration of the basal forebrain cholinergic nuclei is due to the loss of trophic factors secondary to a reduction in the number of cortical neurons. We have used the in vitro application of methylazoxymethanol acetate (MAM) to directly probe this relationship. MAM (15mg/kg ip) or 0.9% NaCl (15mg/kg) was administered to pregnant Sprague-Dawley rats on gestational days 14-15. Brains of the offspring of MAM-treated and control animals were utilized for morphological analysis at 2 months of age, and for neurochemical studies at 2 and 6 months. Anatomically matched sections through the anterior to posterior extent of the brains were rigorously analyzed. The MAM-treated animals demonstrated a 25-35% reduction in cortical cross-sectional area, a 40-65% reduction in cortical volume, and a 50-70% reduction in total cortical neurons in layers II/III, as compared to age and sex matched controls (all values p<.001). In contrast to this extensive cortical lesion, in the 2 month old MAM-treated rat cholinergic markers (ChAT and AChE) were significantly increased in each of the four neocortical regions analyzed. The increases in ChAT levels ranged from 25% to 56% in the neocortex, with significant increases also found in the striatum and hippocampus. Furthermore, this relative, global cholinergic hyperinnervation of the MAM-treated brain was found to be sustained at 6 months of age in all regions examined. It will be of interest to determine if the hypocellular cortex can maintain a cholinergic hyperinnervation over the life span of the MAM-treated rat.

555.9 ORGANIZATION OF NEUROPEPTIDY SYSTEMS AFTER CHOLINERGIC LESIONS OF THE NUCLEUS BASALIS IN RATS. J.W. Unger and W. Lange*. Department of Anatomy, University of Munich, FRG.

Despite the presence of a number of neurochemical alterations, the cholinergic deficit is a consistent finding in brains of patients with Alzheimer’s disease (AD). Since previous studies have shown quantitative changes of cortical somatostatin concentrations in AD patients, as well as rats after lesions of the basal forebrain, our investigation evaluates the response of peptidergic networks after cholinergic deafferentation of several brain regions. 4 weeks and 3 months after bilateral lesions of the nucleus basalis, cell sizes, morphology, density of fibers or terminals are investigated by immunohistochemistry. The distribution of somatostatin and neuropeptide Y neurones in cortex and amygdala remains unchanged after 4 weeks; only minor atrophy of these cells is seen. In addition, galanin fibres in the basal forebrain are spared in lesioned animals. So far, our findings support the hypothesis that cholinergic deafferentation may present only part of the factors that cause widespread neurochemical changes in AD.

(Supported by DFG grant Un 59/2-1)


Because of homology between regions of mouse chromosome 16 and human chromosome 21 we have considered a model of human trisomy 21 (Down syndrome). All trisomy 21 individuals develop neuropathology indistinguishable from Alzheimer’s disease after the third decade of life. It would therefore be of great interest to examine trisomy 16 tissue at various periods of development. MAM-treated rats, a model of neurodegeneration. However, trisomy 16 fetuses die in utero of shortly after birth. We have attempted to maintain trisomy 16 and control tissue by the use of MAM. Hippocampal and neocortical areas were dissected from brains of trisomy 16 and littermate control fetuses after 14-15 days gestation. After incubation with 0.05% trisomy 16 fetuses showed significant loss of tissue in the volume of region. Viability was better than 90% as assessed by e chiidium bromide exclusion. A 16 trisomy 16 was injected into the right striatum of young adult sibling rats. Operated mice were perfused after 2 weeks to 6 months survival. 7 mice with trisomy 16 grafts and 8 mice with control grafts were examined. Beyond violet staining showed surviving grafted tissue in all animals except one. A trisomy 16 graft (7 week survival) which was well integrated with the host brain. Trisomy 16 grafts were smaller than corresponding control grafts of average, but no obvious neuropathology was observed. However, in trisomy 16 grafts at 6 and 6 month survival times selective expression of some Alzheimer’s-associated markers was noted.


Since many of the early pathological and behavioral changes associated with Alzheimer’s disease may be linked to hippocampal damage, we have developed an excitotoxic lesion of bilateral hippocampal disruption as a model. Lesion induction involved a slow infusion of N-methyl-D-aspartate (NMDA) into the lateral cerebral ventricles over 2 weeks, to a total dose of 1 mg NMDA. The animals were behaviorally analyzed using the water maze test (using an index of cumulative escape time - CEM) and then underwent neural grafting of 17-18 day fetal hippocampus as clonk into the lateral ventricles. At time points after the grafting procedures, the animals were again behaviorally tested, and physiological recordings were performed in vitro on the grafts, to assess synaptic integration of the tissue. The goal of the grafts as a trenchly reconnect the CA1 circuitry.

Histological analysis of the lesion showed bilateral CA1 cell loss, with moderate accompanying dentate granule damage. Behavioral analysis at the 14-day survival showed a subset of lesioned animals with behavioral abnormalities (n=15/24; CEM = 1784.3707; mean ± SD, compared to control animals (n=12; CEM = 1033 ± 401; P < 0.01 by t-test). At one year behavioral abnormality was persistent in a subset of animals (n=7/13; CEM = 1764 ± 294). The effects of the grafts on the behavioral abnormalities were mixed, with a subset showing an improvement (n=6/10; CEM = 500 ± 217; P < 0.01 by t-test). Physiological recordings from the grafts in slices showed some synaptic connectivity to the host, but the grafts were in general not well integrated into the host hippocampus. Thus, the integration of the grafts into the host hippocampus and behavioral effects were only moderate. Further characterization will include long-term anatomical and behavioral analysis with suspension grafts. This model may have considerable relevance for the analysis of fetal grafting paradigms in Alzheimer’s disease. Supported by grants from ARDRA, VAMC and B.S. Turner.
556.1 SACCADE EYE MOVEMENTS IN SCHIZOPHRENIA: EVIDENCE FOR IMPAIRED SENSORIMOTOR GATING OF INTERNAL REPRESENTATIONS. D. Hommer, A. Radiant* and S. Nicklow. Department of Psychiatry, VA Medical Center, Washington, DC 20422.

To investigate the proposal that schizophrenia is a disorder affecting the utilization of representational knowledge, we used infra-red topography to measure both visually and internally guided saccades during a task in which, critically, the target was 20° between two points at unpredictable intervals. Next, it moved in a completely unpredictable pattern. Finally, the target resumed the initial pattern of predictable location but unpredictable timing. Horizontal eye movements were recorded from 26 neuroleptic-treated and 8 drug-free schizophrenics, 25 normals and 8 patients with obsessive compulsive disorder. Latency and accuracy of visually-guided saccades and the frequency of adaptive predictive saccades did not differ among the groups. However, when the target motion switched from predictable to unpredictable schizophrenics made significantly more premature saccades directed towards the next target. The frequency of these maladaptive anticipatory saccades significantly correlated with thought disorder. These saccades may result from a failure in the inhibitory gating of premotor commands derived from cortical representations of target motions.

556.2 IMPLICIT MEMORY IN SCHIZOPHRENIA. B.L. Schwartz, R.B. Rosse', and S.J. Deutsch. Psychiatry, VA Medical Center, Washington, DC 20422.

Schizophrenic patients are impaired on explicit recall tests, which depend on higher-order or conceptual processes. The question in this research was whether schizophrenic patients would be impaired on an implicit memory test that also depends on conceptual processes. We examined this issue in two experiments. In Experiment 1, we replicated the deficit in recall obtained in schizophrenic patients. In Experiment 2, we examined implicit memory in schizophrenic patients using two tests, one that depended on conceptual processes (category production) and one that did not (word identification). The results of Experiment 2 showed that performance for schizophrenic patients did not differ from the performance of control subjects in either the category production or word identification test. Implicit memory was unaffected in schizophrenia, irrespective of the nature of the cognitive processes involved in the test. These results suggest that conceptual processes in implicit memory tests may differ from those in explicit memory tests.

556.3 SCHIZOPHRENIA PSYCHOPATHOLOGY FOR THE NEUROSCIENTISTS. W.T. Carpenter, R.W. Buchanan and R. Kirkpatrick. Maryland Psychiatric Research Center, P.O. Box 21247, Baltimore, MD 21228.

Current approaches to diagnosis, proven reliable and valid for many clinical and administrative purposes, are grossly inadequate for the neuroscience-based investigation of schizophrenia. Multiple criteria are drawn from divergent aspects of behavior are poorly suited guides for developing animal models. An alternative approach to the use of criteria is to subdivide schizophrenia into discrete domains of psychopathology. Five such domains have been defined: expressive symptoms, cognitive symptoms, affect, default functions, and neurologic manifestations. The neuroscience application of domains can be illustrated in two areas: 1) defining the phenotype for linkage studies; 2) establishing animal models for specific psychopathologic attributes. The authors believe that this shift in focus from diagnosis to domains will facilitate neuroscience investigations of schizophrenia by providing more relevant and applicable psychopathologic targets. This approach may diminish the false negative ascertainment in pedigree-based studies and decrease the speculative inference of animal models to schizophrenia. An animal model of social affiliation, asedation, or attentional dysfunction will require less speculative inference, and the relevance to schizophrenia will be more straightforward.


The degree of cortical folding, as measured by the gyri-convexity index (GCI), was assessed to test the hypothesis that sulcal/gyrall patterns in the temporal lobe differed between schizophrenic and normal brains. The GCI's were measured separately for the pole, dorsal, medial and lateral regions of the temporal lobe of 10 normal, 10 schizophrenic brains with leukemias and 6 non-schizophrenic brains with leukemias. The brains came from the Yakovlev Collection.

Both sets of leukotomized brains had a decrease in folding in the dorsal (Sylvian) region compared to normals, but an increase in relative amount of folding in the left medial cortex at the level of the anterior hippocampus. The GI's in the schizophrenic brains differed from the others in having a decreased degree of folding in the left medial cortex at the level of the amygdala, suggesting specific anomalies in this region. The medial cortex is from the amygdala to the medial bank of the occipito-temporal sulcus and includes much of the entorhinal cortex.

Supported by NIH 45594.

556.5 AGE-RELATED CT MEASURES IN SUB-GROUPS OF SCHIZOPHRENIC PATIENTS. Arthur Kling, Alan Steinberg, Peter Lucas, Neena Sachivala, Ken Tachiki, Harald von Scotti, R. F. Ritzmann. Sepulveda VAMC, UCLA. Los Angeles, CA 91433.

Seventy-two schizophrenic patients and 41 controls had head CT scans. Patients were subtyped according to DSM III-R criteria. Measures were made of the area of the lateral ventricles and the amount of cortical atrophy in various brain regions. VBR differentiates between genoid and frontal/uni- differentiated schizophrenic subgroups between the ages of 30-49. Total brain atrophy differentiates residual/undefined schizophrenics from normal/schizophrenics and/or controls after the age of 50. After the age of 40, atrophy in the sylvian region differentiates between controls and controls. Regardless of sub-classification. To assess the possibility that alternative diagnostic classification systems would yield more meaningful data, a group of patients was classified according to three alternative typologies: Positive/Negative Type I/Type II; Deficit/Nondeficit. No significant correlation with CT findings was generated through these alternative classification methods. Supported by the research service. Sepulveda VAMC.

556.6 REGIONAL VARIATION OF NEUROANATOMICAL BRAIN ABNORMALITIES IN SCHIZOPHRENIA. M. S. Kytelowski, R. Coppola, K. F. Torey, D. A. Wehnerger. NIH Neuroscience Center, St. Elizabeths, Washington, DC 20032.

Structural brain pathology encountered in schizophrenic patients poses a question whether the abnormalities are centered predominately at the limbic-prefrontal system. We answered this question by comparing the size of the septum with the size of the cuneus and the socalled pattern of the occipital lobe. The latter remains a unique brain region in that it has not been implicated in schizophrenia. Subjects were 19 pairs of monozygotic twins discordant for schizophrenia (range of discordance period was 1-24 yr). For all pairs MR images were obtained using identical T1 weighted spin echo pulse sequences (TR/TI=600/20). Sagittal cuts (5 mm thick) were analyzed to compare the size of the septum, cuneus, and the course of the medial occipital sulci. The variance zone for the parieto-occipital and retrolateral cuneus, the slopes of the right and left retrolateral cuneus, and the size of the cuneus were distinctly similar between and between the two groups. Thus, in smaller (11.6%) increase of the size of the septum in the impaired group. Two-sample sign test confirmed a high significance of this difference (p=0.002). Thus, the impaired twin could be differentiated by increased septal area, but not by the anatomy of the occipital lobes within the same geometrical space of the brain.
Evidence for diffuse gray matter abnormalities in schizophrenia. B.B. Zipursky, K.O. Lim, A. Mattfeldt. Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Palo Alto, CA. A Magnetic Resonance Imaging (MRI) study was undertaken to determine its extent to which the greater ventricular and sulcal volume found in schizophrenic patients is due to differences in gray versus white matter volume.

Axial MRI scans were obtained using a GE Sigma 1.5 T scanner from 22 patients meeting DSM-III-R criteria for schizophrenia and 20 healthy community volunteers. All subjects were right-handed male veterans, 20 to 45 years of age. Volume data, which were corrected for subject thickness, were determined for all scans for axial images and subtracted from the mean (± SEM) volume. No age-related differences were found between the two groups.

Within the cerebral cortex of the community volunteers, percentage gray matter volume but not percentage white matter volume was significantly correlated with age (r = .26, P < .001). After correcting for the effect of age, the schizophrenic group was found to have significantly lower cortical gray matter volume than the control group (mean Z = 1.68, P = .001). Significant differences in percentage gray matter were found in all regions. No significant differences were found in percentage white matter in the cortex as a whole or in any of the cortical subregions.

These results are consistent with the view that relative gray matter volume is abnormally low throughout the brain in patients with schizophrenia as a group. It remains to be established whether specific regions are disproportionately affected.

Supported by MH 30854, NARSAD and the Department of Veterans Affairs.

Growth promoting agent found in schizophrenic CSF changes neuroblastoma cell properties. S. Shimakura1,2, J.P. Schwartz2, and J.R. Stevens1. 1) NIMH, St. Elizabeths Hosp., Washington, DC; 2) C.B. CB, NINDS, NIH, Bethesda MD 20892. This study was undertaken to look for a transmissible agent produced in tissue culture by a small minority of schizophrenic patients. The human neuroblastoma cell line SH-EP (NB) was incubated for 5 days with cerebrospinal fluid (CSF) from patients with schizophrenia (Sch) of 2-22 years duration (mean 8.9 yr) from St. Elizabeths Hosp., Washington DC, or various types of control CSF, after which cells were passaged at 2 week intervals.

After 2-6 months culture, NB cells which had been treated with fresh CSF from 12/12 Sch patients showed 30-210% higher density growth than 15 control CSF-treated NB cell cultures (p<.01). The growth promotion could be transmitted by cell-free media from Sch CSF-treated cells. Seven of 9 Sch CSF- but only 1 of 13 control CSF-treated cultures showed increased size and/or number of colonies upon growth in soft agar. NB cells treated with Sch CSF showed changes in their intracellular neurofilament distribution by immunocytochemistry, using several different antibodies.

Experiments are underway to identify the agent responsible for the cell transformation.


Few publications have appeared regarding the levels of free amino acids in cerebrospinal fluid (CSF) of schizophrenic patients. We report the results of the determination of 21 free amino acids levels in CSF of 21 non-patient controls (1 woman, 2 men; mean age 54.1, 41-113.7 years) and seven paranoid schizophrenic patients (4 women, 3 men; mean age, 26.7, 81). The samples of CSF were taken between 8 and 11 a.m.

The patients and controls had been at bed rest for at least 8 hours and fasting for 14 hours. Lumbar puncture was made between 14 and 11. The amino acids analysis was performed by HPLC. The schizophrenic patients had higher (p<0.05) CSF concentrations of glutamate than controls (57.4 ± 8.1 and 154.5 ± 115.7 amole/liter, respectively). The levels of the remaining 20 free amino acids did not differ from control values. No significant correlation was detected between age, sex, and content of amino acids in CSF.

We could not confirm the findings of Rijkerstraat et al. (Br.J.Psychiat. 141: 276, 1985) that CSF levels of histidine were elevated in schizophrenic patients. Similarly we did not find any significant increase in the concentrations of CSF alanine, glycine, leucine, and phenylalanine as reported by Roveley et al. (J.Biol.Psychiat. 22: 413, 1957).

PET/FDG localization of metabolic changes in schizophrenia. G.K. Thaker, A. Summaria1, M.B. Yahr1,2, T.N. Chase, C.A. Tannin,3 Maryland Psychiatric Research Center, University of Maryland at Baltimore, Baltimore, MD 21228

Regional analysis of cerebral glucose utilization may serve to localize areas of psychopathology in schizophrenic brain. PET/FDG studies were carried out in drug-free schizophrenic patients to test the hypothesis that areas of altered metabolism would parallel those brain areas in animals affected by the psychotomimetic PCP. Twelve young schizophrenic subjects with mixed florid psychotic symptoms and matched normals were scanned using "FDG-2-deoxyglucose on the NeuroPET scanner, five transverse planes in each subject were analyzed blindly using ROIs matched to a human brain atlas. Results showed no differences between schizophrenia patients and normals in the neocortical or extrapyramidal areas, but a significant decrease in glucose metabolism in limbic structures in subjects with schizophrenia. Specifically, the rate of 18F-FDG uptake was decreased in hippocampus and anterior cingulate. This distribution of metabolic changes paralleled the metabolic alterations in rat brain following PCP and MK-801. Differences in schizophrenic subgroups will be reported.


Subclassification of schizophrenic symptoms leads to the identification of discrete neural circuits associated with specific domains of psychopathology. We have studied two of these domains, positive psychotic symptoms and primary, enduring negative or deficit symptoms for discriminating neuroanatomical, neuropsychological, and electrophysiological characteristics. Substantial evidence now exists to suggest that different neural circuits are involved in the production of these symptoms. Limbic circuits appear to mediate positive symptoms and distinguish schizophrenic patients from controls. In contrast, deficit is distinguished from non-deficit schizophrenia by significantly reduced regional glucose utilization (mg glucose/100 g tissue/min) in the thalamus (deficit: 8.1 ± 0.8; non-deficit: 10.4 ± 0.7) and in multiple regions of the frontal and parietal cortices. Deficit patients also exhibit significantly increased impairment on neurological measures of parietal lobe function (deficit: 3.29 ± 1.10; non-deficit: 1.47 ± 1.12) and significantly increased voluntary saccadic latency (sec's) (deficit: 441 ± 94; non-deficit 351 ± 70), an eye-tracking measure sensitive to frontal and/or parietal lobe impairment. These data implicate the involvement of the thalamus and frontal and parietal cortices in the production of deficit symptoms.


In this study, we attempted to determine if an acute dose of dextroamphetamine might have positive effects on affect and cognition in schizophrenic patients maintained on haloperidol. We based this premise on the rationale that dopamine type 1 receptors in frontal cortex might be indirectly stimulated by amphetamine, resulting in improved affect and cognition, while the potential psychomotor effects of amphetamine stimulation of subcortical type II receptor sites would be prevented by haloperidol blockade. In a double-blind placebo crossover study, 21 patients with chronic schizophrenia received a single oral dose of amphetamine at 25 mg/kg. All patients were receiving 4 mg/kg daily of haloperidol. Six patients showed significant improvements in their ability to have improved in terms of affect and engagement with the environment. Improvement was associated with enlarged cerebral ventricles and also with increases in binding from pheno to placebo treatment. Amphetamine also improved some parameters of performance on the Wisconsin Card Sorting test of concept formation and set shifting, though it did not result in change in memory or attention. The possibility that a dopamine agonist in conjunction with a dopamine type II receptor antagonist might prove efficacious in schizophrenia may have implications for understanding the neural systems involved in this disease.

Paroxetine is the ligand of choice to label the serotoninergic uptake sites in human brain (Laruelle, M., Biol. Psychiatry, 24:299, 1988). Because of the implication of the serotonin system in mood and suicide, we performed post mortem saturation analysis of \( \text{[H]paroxetine} \) (0.065 to 2nM) specific binding (defined as the binding displacable by 1μM citalopram) in the frontal pole of patients suffering from schizophrenia (n=10), chronic schizoaffектив illness (n=5), non psychotic suicide (n=8) and matched controls (n=10). Samples from cocaine addicts were also analysed (n=4) due to the reported toxicity of chronic cocaine administration for serotonin uptake sites in rats (Terry L.M., Soc. Neurosci. Abst. 32:11, 1989). Kd values did not exhibit significant differences between the groups. Bmax values were significantly different (ANOVA, p<0.05): controls 84±13 fmol/mg protein (mean±SEM), schizoaffectives 81±8 fmol/mg, schizophrenics 61±10 fmol/mg, and cocaine addicts 64±10 fmol/mg. Post-hoc analysis showed a significantly lower Bmax in schizophrenics and suicides compared to controls. These results suggest an abnormality of the serotonin presynaptic system in schizophrenia and such a deficit may support in human the notion of toxicity of cocaine toward these terminals as described in rats.

556.15 ELEVATED D2 DOPAMINE RECEPTOR DENSITY IN 26 SCHIZOPHRENIC PATIENTS: L. Yue, D.F. Wong, H.N. Barger, R.P. Dansky, The Johns Hopkins Hospital, Medical Institutions, Baltimore, MD 21205.

Utilizing 11C-methylspiperone positron emission tomography (PET) striatal D2 dopamine receptor density (Bmax) was compared in 26 chronic schizophrenic subjects, 21 of whom were drug-naive at the time of scan (and an additional 5 with minimal neuroleptic pretreatment). All patients satisfied DSM-III-R criteria for chronic schizophrenia illness. The average age was 37.65 +/- 3.5 years. The average illness was 4.63 +/- 0.89 years (without proctode) and 7.22 +/- 1.29 years with proctode. Dopamine receptor density (Bmax) in schizophrenic subjects (32.35 +/- 17.89) was significantly elevated when compared to control subjects (15.46 +/- 9.26). Bmax overlapped with normal controls in 11 of 16 samples. Clinical and neuropsychological test results were then compared with Bmax values and will be presented.


Use of phenocyclidine (PCP) produces behavioral effects resembling schizophrenia, which may be due to activation of CNS dopaminergic systems. Using HPLC-EC, endogenous dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) were measured in superfusates from striatal slices of PCP (0.23 μM) treated and untreated rats. Slices were placed in each of two superfusion chambers and were superfused for 1 min with Krebs' buffer for 60 min. Subsequently, chambers were reperfused for 60 min with Krebs' buffer or with buffer containing PCP for 3x10^-5 M, followed by control buffer for a variable period (5, 30, 60 and 120 min) followed by a second, 60 min exposure to PCP 3x10^-4 M. Initial exposure to PCP resulted in a peak increase in the concentration of DA and DOPAC (120 and 450 pg/mg/ml, respectively). Despite the continued presence of PCP, DA and DOPAC concentrations returned to basal levels. Perfusion to PCP diminished the subsequent response to PCP (70% of control for DA, 40% for DOPAC). However, DA and DOPAC concentrations were not different from control when superfusion for 30 or 60 min period, respectively, was interrupted between PCP exposures. Therefore, the diminished response was not due to depletion of the releasable pool of DA, but to a downregulation of D2 dopamine receptor response of the dopaminergic neuron. Supported by NIMH MH42934, NIMH Fund and NIDA Contract 271-87-8133.

556.14 Abstract Title: A COMPARISON OF CHRONIC SCHIZOPHRENICS WITH INTERICTAL PSYCHOTICS

Author(s): Name: Estelle Toby Goldstein, M.D., Sheldon H. Perskonias, M.D., Beryl Syley, M.D., Linda Hauser, M.D.

Patients with the interictal psychosis of partial complex epilepsy (n = 18) were compared with chronic schizophrenia (n = 17). The patients had been followed in the inpatient and outpatient units of the Virginia Commonwealth University Administration Medical Center Psychiatry Service. Epileptic patients did not differ significantly from schizophrenics in age at index hospitalization (Mean ± SD = 45 ± 9.4 yr. vs. 43.4 ± 10.8 yrs., respectively; t = 0.58, df = 33, N.S.), although schizophrenic patients were about ten years younger at age of DSM-III diagnosis than the epileptic patients, and the latter had a broader range of age at which diagnosis was made (Mean ± SD = 23.2 ± 6.4 yrs.; 32.5 ± 12.5 yrs., respectively, t = 2.34, df = 28, p < 0.01). Schizophrenic patients became psychotic at an earlier age than epileptic patients (Mian ± SD = 22.6 ± 6.8 yrs. vs. 33.3 ± 10.3 yrs., Student's t = 3.03, df = 43, P < 0.005).

The epileptic group was more likely to have had a documented history of head injury (X² = 8.31, P = 0.003) than the schizophrenic group. Documentation of other major injuries, however, did not differ significantly between the two groups (X² = 5.5, M.E., N.S.).

The medical histories of these two groups were not notably different. Two patients in the chronic schizophrenic group had a family history of schizophrenia (maternal side), compared to no family history in the epileptic group. Family history for non-psychotic psychiatric illness (alcohol abuse, depression, etc.) was equally represented in both groups (live in each group).

Additional comparisons of these syndromes are made, with special reference to clinical features of the psychosis, other behavior disturbances and treatment response.


Previous studies (Reynolds G., Nature, 305:527, 1983) have reported a higher concentration of dopamine (DA) and, to a lesser extent, homovanillic acid (HVA), in the left compared to right amygdalas of patients with schizophrenia. In an attempt to replicate these findings, we measured DA, HVA and DOPAC in the right and the left amygdalas of schizophrenics (n = 6), drug-naive controls (n = 6) and matched controls (n = 6). For DA, results were (Mean±SEM) as follows: control: 0.83±0.36mg/gm protein, left 0.43±0.63mg/gm, right 0.46±0.57mg/gm; schizophrenics: right 0.46±0.35mg/gm, left 0.46±0.04mg/gm; cocaine addicts: right 1.70±1.19mg/gm, left 1.04±0.45mg/gm. Neither the diagnosis factor, the hemispheric side factor, nor the interaction between these factors showed a significant effect when analysed with a 2 factor repeated measure ANOVA. Results of HVA and DOPAC levels were also negative. Nevertheless, the schizophrenic group was the only group that showed higher DA levels in the left side compared to the right. This was also true for HVA. More samples should be studied before a conclusion could be reached.


Pedunculopontine nucleus (PPN) neurons, along with adjacent cholinergic nuclei, have been implicated in the modulation of sleep and movement. The cholinergic cell group appears to be the generator of the P1 auditory evoked potential (Buchwald et al., 1995) which in turn fails to habituate in schizophrenia (Freeman et al., 1983). Cholinomimetics produce sleep and affective disturbances similar to schizophrenia and a role for cholinergic hyperactivity has been proposed in this disorder. Human brain tissue was obtained from five diagnosed schizophrenics (age 62±10) and from five control subjects (age 60±5). One half of the mesopontine brainstem was fixed and frozen sections processed for NADPH diaphorase histochemistry (which labels cholinergic mesopontine cells) and to determine choline acetyltransferase (AChE) activity (control) in these samples. The cell number and size of cholinergic PPN and lateralmost tegmental (LDT) nuclei, as well as the nonadrenergic locus coeruleus (LC) were measured. Wilcoxon Test P<0.05.

<table>
<thead>
<tr>
<th>Cell number</th>
<th>PPN</th>
<th>LC</th>
<th>LDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.50±5.621</td>
<td>11.05±4.761</td>
<td>6.38±5.736</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>18.27±5.822**</td>
<td>11.49±2.956</td>
<td>12.26±7.774</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell Size (μm)</th>
<th>PPN</th>
<th>LC</th>
<th>LDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>517±167</td>
<td>1035±105</td>
<td>652±37</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>517±44</td>
<td>781±106**</td>
<td>553±58</td>
</tr>
</tbody>
</table>

These preliminary findings suggest the presence of a greater number of cholinergic PPN neurons in the brains of schizophrenics. Such a hyperactivity has been consistent with the proposed but ill-programmed cell death in this disease (Finberg 1982, perhaps leading to cholinergic hyperactivity. The reduced cell size present in LC could be a result of neuropilic treatment, and may contribute further to the distribution of PPN in this illness.)
Mental Illness: Schizophrenia

Three brain antigens in schizophrenia

E.G. Berton, C.A. Kaufmann, J.E. Kleinman, M.F. Casanova*, L. Gleeson, P. Davies* Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY (U46)

We have described a panel of monoclonal antibodies for schizophrenia brain studies (Brain Res 1989;500:379). Detailed review of 3 of these antibodies is now reported.

Anti Ep10 binding to schizophrenia (sch) brain homogenates was increased compared to controls (ccm) (U=18, p<.001, n=9 each). This increase appeared to be globally distributed in 5 brain regions. Tissue staining with Ep10 indicated a pattern typical for synaptic antigens. Immunoblot analysis revealed a reactive band at about 38 kb. A similar protein band immunoprecipitated by Ep10 was reactive with an antibody against synaptophysin. This, or a similar synaptic molecule may be elevated in the sch brain. Ep10 is sensitive to sch cytokine was decreased compared to ccm (U=2, p<.01, n=9 each). Tissue staining showed neuronal cell bodies as well as apical and basilar dendrites. Antibody Ep7 reacts to temporal cortex from male cases of schizophrenia reduced compared to male ccm (U=3, p=.05, n=8 each, 4 ccn). In tissue sections Ep7 appeared to stain distal axonal profiles.

Biochemical studies on the Ep1 and Ep7 antigens are in progress. These preliminary results using the Ep antibodies indicate that several distinct molecular abnormalities may be present in the schizophrenia brain. Supported by NARSAD, NRC Canada and Metropolitan Life.

Response latency structure in schizophrenia as related to dopamine overstimulation theory. M. Lysan and N. Lysan, Center for Schizophrenia Research, Department of Psychiatry, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Previous investigations have shown the presence of increased switching between responses and increasing stereotypy of responses in schizophrenic patients even on a very simple decision task (Lysan et al, 1986; Lysan and Gerlach, 1988). This was interpreted as support for the effects of dopaminergic overstimulation effects on response sequencing as predicted by Lyss and Robbins (1975) and Robbins and Watson (1981). If this interpretation is correct then the response latency structure should be more consistently patterned in schizophrenic patients, with resulting increases in number of fixed patterns and in the variety of patterns found to be repeated.

The present study examines the structure of response latency patterns between two-choice decisional responses produced by schizophrenic patients (N=17) and age, sex, and educationally matched normal control subjects (N=17) used by Lyss et al. (1986). Sequential time range patterns in the latencies were scored by a computer program (Mannagon, M.S., Beca de los Condores de Trabajo 1988) which selected only patterns which occurred at least 3 times with a variability of interspontaneous latencies having a probability of P<.0001.

Results showed that schizophrenic patients had significantly more number of individual "outlier" latencies that prevented some patterns from being significant. These results give further support to the twin principles of increased switching and final stereotype as important markers in schizophrenia behavior.

Dopamine D1, but not D2, receptor blockade reverses amphetamine-induced changes in auditory gating. K.E. Stevens, L.I. Fuller*, and G.M. Rose. Dept. of Pharmacology, UCHSC; and VAMC, Denver, CO 80262

The N40 auditory evoked potential recorded in response to the second of a closely spaced pair of stimuli is reduced compared to the first. This gating phenomenon is disrupted by administration of amphetamine. In addition, both conditioning and test evoked potential amplitudes are reduced compared to unmedicated trials. These changes are reversed by haloperidol. Here we report the results of selective dopamine receptor blockade on the amphetamine-induced changes. Rats were chronically implanted with an recording electrode at the vertex. Following recovery, the N40 potentials in response to paired, 71 dB click stimuli (presented 0.5 s apart, exaggeratedly repeated over several days. Animals with normal gating were then given amphetamine (1.83 mg/kg, i.p.) to confirm drug-induced disruption of gating. Following this, animals were given either the D1 antagonist, 23200 (0.5 mg/kg, i.p.) or the D2 antagonist, sulpiride (40 mg/kg, i.p.) and the effects on N40 observed. Blockade of D1 receptors reversed the amphetamine-induced changes in N40, yielding responses not different from the unmedicated state. In contrast, the D2 antagonist did not affect amphetamine-induced changes in N40. Administration of the antagonists alone had no effect on normal responses. Thus, changes in N40 amplitude, and the concomitant disruption of gating, by induced amphetamine appear to be mediated through a dopaminergic, D1 receptor mediated, mechanism. (Supported by P50 MH44212-01)

Abnormal growth of fibroblasts from schizophrenic patients is not due to acute neuroleptic effect. L. Laev, R. Reddy*, S. Muthukrishnan and S.P. Mahadik. Division of Neuroscience, NYSPI & Columbia Univ., N.Y., N.Y.

We have observed abnormal growth and morphology of skin fibroblasts from schizophrenic patients compared with normal controls. Cultures were established from skin biopsies of 14 schizophrenic patients and 12 age-matched normal subjects. Fibroblasts cultured from these patients showed differential growth and morphology as follow:

- Initial growth: fibroblasts from schizophrenic patients took considerably longer to establish than those from controls.
- Established cultures were obtained from schizophrenics after 2-4 months for schizophrenic patients. Rate of growth: doubling time for fibroblasts from schizophrenics was markedly longer than that of normals.
- Morphologic differences: fibroblasts from normals showed typical unipolar, long, spindle-like appearance and unidirectional orientation. Fibroblasts from schizophrenics exhibited random sizes (shorter, flatter) and orientation.

Since all of these patients were on neuroleptic treatment, confounding effects of acute neuroleptics on these growth characteristics are difficult to resolve. To address this issue, fibroblasts cultures from skin biopsies of patients (N=6) off-drug at the time of biopsy, normals (N=6) challenged in culture with haloperidol and normal established cultures (N=4) challenged with haloperidol in culture were studied. The initial growth of fibroblasts off-drug was improved but was still not identical to normals. Rate of growth and morphology were still abnormal. There was no effect of haloperidol challenge of skin biopsy of normals or normal fibroblast culture on any of the growth parameters. Data indicate that acute haloperidol has marginal effect on initial growth only and abnormalities observed in patient fibroblasts are probably related primarily to disease process. Further studies with skin biopsies from never medicated patients will help to understand interaction of treatment with disease process.


The N40 auditory evoked potential recorded in response to the second of a closely spaced pair of stimuli is reduced compared to the first. This gating phenomenon is disrupted in schizophrenia, and can be disrupted by amphetamine in humans and rats. This study evaluated the effects of intraventricular kainic acid administration on the N40 to intraventricular kainic acid. The dose of kainic acid was 0.5-0.7 mg kainic acid. Vertex recordings were obtained 2 weeks to 4 months after infusion, and results were compared with 15 unlesioned rats. The kainic acid treated rats had significantly lower mean conditioning N40 amplitudes and higher conditioning-testing ratios. These parameters did not vary over time in either lesioned or unlesioned animals. Pyramidal cell loss in region CA3 of the lesioned rats varied between 41-88%. However, the extent of the damage to CA3 did not correlate with any N40 parameter. (Supported by P50 MH44212-01)

Partial hippocampal damage abolishes facilitative effect of stress on simple learning: Further analysis of an animal model of schizophrenia. K.H. Seybold, B. Campton*, J. Kendall* and R. Johnson* Dept. of Psychology, Dept. of Psychology, Grove City College, Grove City, PA 16127 and Slippery Rock University, Slippery Rock, PA, 16057.

A putative animal model of schizophrenia has been developed based on hippocampal neuropathy described in human schizophrenia. Preliminary analyses (Seybold et al, 1989) revealed that slight hippocampal damage in rats produced a facilitation of learning on the Morris Water Maze task similar to the effects found in schizophrenic humans. The present experiment evaluated the effects of stress, which facilitates performance in normal subjects, on N40 and also on other auditory evoked potentials on the Morris Water Maze task. Preliminary analyses (Seybold et al, 1989) revealed that slight hippocampal damage in rats produced a facilitation of learning on the Morris Water Maze task. Preliminary analyses (Seybold et al, 1989) revealed that slight hippocampal damage in rats produced a facilitation of learning on the Morris Water Maze task. Preliminary analyses (Seybold et al, 1989) revealed that slight hippocampal damage in rats produced a facilitation of learning on the Morris Water Maze task.